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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **311th Meeting** *of the Biochemical Society was held in the Department of Biochemistry, St Thomas's Hospital Medical School, London, S.E.* 1, *on Friday,* 24 *October* 1952, *when the following papers were read:*

COMMUNICATIONS

Malonate Inhibition of Succinic Dehydrogenase. By M. B. Thorn. (*Department of Biochemistry, St Thomas's Hospital Medical School, London, S.E.* 1)

The mechanism of competitive inhibition of succinic dehydrogenase by malonate (Quastel & Wooldridge, 1928) may be represented:

$$E+S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_3}{\rightarrow} E+\text{products}, \quad K_m=\frac{k_2+k_3}{k_1},$$

$$E+I \underset{k_5}{\overset{k_4}{\rightleftharpoons}} EI, \quad K_i=\frac{k_5}{k_4},$$

where E is succinic dehydrogenase, ES the enzyme-succinate complex, and EI the enzyme-malonate complex. 'Relative affinity' means the ratio K_m/K_i, which is approximately equal to the ratio $[S]/[I]$ at 50 % inhibition of the catalysed reaction, provided that $[S]$ is much greater than K_m.

Conflicting values have been obtained for the relative affinity of succinic dehydrogenase for succinate and malonate. Krebs & Johnson (1948) obtained a figure of 10 with minced pigeon-breast muscle. Potter & DuBois (1943), using rat-liver homogenate, report a value of 50. Thorn (1951), using heart-muscle preparation with methylene blue and cyanide, obtained figures from 4·7 to 7·5 with methylene blue from 4×10^{-4} to 10^{-3}M. Krebs, Gurin & Eggleston (1952) found a value of 60 with yeast preparations.

The work to be reported was carried out using heart-muscle preparations (Keilin & Hartree, 1947). Succinic dehydrogenase activity was measured in two ways. The first method was by reduction of $K_3Fe(CN)_6$ in the presence of cyanide, using the Spekker absorptiometer (Ilford 601 filter) to measure the rate of decrease of optical density in the region of 400 mμ (essentially the method of Slater & Bonner, 1952). The second method was manometric, using methylene blue in the presence of cyanide. Succinic oxidase activity was measured manometrically.

K_m and K_i were determined (Lineweaver & Burk, 1934) using $K_3Fe(CN)_6$. K_m of succinic dehydrogenase in six preparations was between $2\cdot5\times10^{-4}$ and $5\cdot3\times10^{-4}$. K_i for malonate was between $5\cdot4\times10^{-6}$ and $9\cdot8\times10^{-6}$. Corresponding values gave calculated K_m/K_i ratios of about 50. In one preparation K_m/K_i, determined directly, was 58 for succinic oxidase, and 60 for succinic dehydrogenase by the $K_3Fe(CN)_6$ method. This agreed with a value of 57 calculated from separate determinations of K_m and K_i. When the ratio was determined for succinic dehydrogenase by the methylene-blue method, it varied from 6·5 to 24 with a 20-fold increase in methylene-blue concentration.

These results can be explained according to the theory worked out by Slater & Bonner (1952) for fluoride and phosphate. Values of the constants k_1, k_2 and k_3 were determined, and agreed substantially with those of Slater & Bonner. The results will be discussed.

The author gratefully acknowledges his indebtedness to Dr E. C. Slater and Dr W. D. Bonner for access to a draft of their paper before publication.

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The Adeninenucleotide Specificity of Oxidative Phosphorylation. By E. C. SLATER and F. A. HOLTON. (*Molteno Institute, University of Cambridge*)

Although recent studies have shown that adenosinediphosphate (ADP) is often phosphorylated by respiring tissue preparations much more rapidly than adenosinemonophosphate (AMP) (Slater, 1950; Barkulis & Lehninger, 1951; Kielley & Kielley, 1951; Lindberg & Ernster, 1952), they have not determined unequivocally the adeninenucleotide specificity of the oxidative phosphorylation reaction itself. In view of the rapid phosphorylation of AMP found in earlier work, the possibility remained that the oxidative phosphorylation system is not completely specific for ADP, but reacts slowly with AMP. Once ADP was formed by this reaction, it would react in preference to the AMP. This possibility has been tested by measuring the formation of energy-rich phosphate groups ($\sim$P) by a sensitive enzymic method (Slater, 1951), during the oxidation of α-ketoglutarate by a very dilute preparation (0·12 mg. protein/ml.) of cat heart-muscle sarcosomes (mitochondria). In contrast to liver mitochondria, cat-heart sarcosomes possess only very slight myokinase activity, in the presence of fluoride and AMP, two inhibitors of myokinase. The following results have been obtained:

(1) The relative rates of oxidation of α-ketoglutarate with various additions were: no addition, 46; +hexokinase+glucose, 75; +ADP, 100; +ADP+hexokinase+glucose, 100. The high rate of oxidation in the absence of ADP, especially with added hexokinase and glucose, is presumably due to the presence of endogenous $\sim$P (Green, Atchley, Nordmann & Tepley, 1949; Kielley & Kielley, 1951). With the enzyme concentrations used, the amount of endogenous $\sim$P was below the limits of detection by the analytical procedure. In the presence of hexokinase, the P:O ratio was 1·24 without added ADP and 2·72 with added ADP.

(2) When AMP was the only adenine nucleotide added, no $\sim$P ($<0{\cdot}02$ μmole) was detected, either in the absence or presence of hexokinase, even after 45 min., when 1·1 μmoles of hexosemonophosphate were formed in the presence of hexokinase. With ADP, the following values for $\sim$P were found after 1, 3 and 10 min.: 0·03, 0·08, 0·28; corresponding to a P:O ratio of 2·4 (cf. 3·0 with added hexokinase).

These results show that AMP is not directly phosphorylated in the oxidative phosphorylation reaction itself. Its rapid phosphorylation by respiring liver mitochondria must depend upon the formation of ATP from endogenous ADP, followed by the myokinase reaction (cf. Kielley & Kielley, 1951). Our conclusions are in opposition to those of Lindberg & Ernster (1952).

This work was supported by personal grants from the Agricultural Research Council.

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Transfructosidation by Mould Invertase Preparations. By J. EDELMAN (*Research Institute of Plant Physiology, Imperial College, London, S.W.* 7) and F. J. BEALING (*Department of Biochemistry, University of Sheffield*)

Enzymes hitherto regarded as invertases appear to act primarily as transglycosidases (Blanchard & Albon, 1951; Bacon & Edelman, 1951*a*; Edelman & Bacon, 1951; Bealing & Bacon, 1951; Fischer, Kohtès & Fellig, 1951; Bacon, 1952). The use of radioactive glucose has further elucidated the reactions involved.

Samples (3 μl.) of a digest incubated at 20° and containing 10 mg. radioactive glucose, 15 mg. inactive sucrose, 0·12 ml. 0·011M-acetate buffer pH 5·0, and 0·08 ml. mould invertase preparation (from *Penicillium spinulosum*, see Bealing & Bacon, 1952) were chromatographed on paper sheets (Partridge, 1948). Using a thin end-window Geiger-Müller counter directly on the paper, the counts/min. (c/m) of the glucose spots were: after 3 min. incubation 4133, 30 min. 3350, 60 min. 3439, 120 min. 3624. The corresponding c/m of the sucrose spots were 218, 551, 546 and 340, and of spot α (?trisaccharide consisting of two fructose and one glucose units, see Bealing & Bacon, 1952) were 8, 91, 212 and 377. Spot β (?tetrasaccharide consisting of three fructose and one glucose units) was present in the 60 and 120 min. samples, the c/m being 10 and 62 respectively. Recoveries of the c/m applied were 95–97%. At 120 min. these values represented 16·8 (sucrose),

11·4 (trisaccharide) and 8·8 (tetrasaccharide) c/m/μg. combined glucose, assuming each spot to contain only the postulated oligosaccharide. Two-dimensional chromatography with hydrolysis of oligosaccharides after the first development (Bacon & Edelman, 1951*b*) showed that only the glucose parts of the molecules were labelled.

Isolation of glucose-labelled sucrose from a similar digest was accomplished by adding 25 mg. carrier sucrose after 30 min. and separating the sugars on a charcoal-Celite column (Whistler & Durso, 1950); crystals obtained were identified as sucrose from X-ray powder photographs. The c/m/μg. after crystallization and recrystallization were 3·48 and 3·64 respectively.

These results support the hypothesis that mould invertase transfers fructose residues to suitable acceptors, including water, glucose and sucrose (Bealing & Bacon, 1951); transfer to glucose gives sucrose, and further transfer to glucose-labelled sucrose gives similarly labelled higher saccharides (spots α, β). Thus addition of glucose to an enzyme-sucrose digest decreases the rate of sucrose breakdown owing to the increased rate of reformation of the initial substrate; this would explain the long-known inhibition of mould (taka-) invertase by glucose (see Neuberg & Mandl, 1950).

We thank Dr H. K. Porter for the gift of radioactive glucose, and Dr A. J. E. Welch and the Royal Society for the X-ray photographs of sucrose.

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The Conversion of Naphthalene to 1:2-Dihydronaphthalene-1:2-diol monoglucuronide in the Rabbit. By E. D. S. Corner, F. S. Billett and L. Young. (*Department of Biochemistry, St Thomas's Hospital Medical School, London, S.E.* 1)

From the urine of rats and rabbits dosed with anthracene, Boyland & Levi (1935, 1936) isolated 1:2-dihydroanthracene-1:2-diol and also the monoglucuronide of this compound. 1:2-Dihydronaphthalene-1:2-diol is excreted in the urine of rats (Young, 1947) and rabbits (Booth & Boyland, 1949) following the administration of naphthalene, but the excretion of a conjugated form of this diol has not hitherto been reported.

In the present work it was found that although there was little increase in the ethereal sulphate content of the urine of rabbits after they had been dosed with naphthalene, there was a marked increase in the glucuronide content of the urine. This led to an investigation of the possibility that the urine of the dosed animals contained a glucuronide of 1:2-dihydronaphthalene-1:2-diol. From the urine of these animals it was found possible to obtain a powder which contained glucuronide. Attempts to separate a pure glucuronide from this powder always resulted in the formation of gums which could not be induced to crystallize. When the powder was treated with ethereal diazomethane followed by acetylation of the product, however, a crystalline compound was obtained, the analysis of which corresponded to that of the tetra-acetyl derivative of 1:2-dihydronaphthalene-1:2-diol monoglucuronide methyl ester; m.p. 209°; $[\alpha]_D^{20°} = +94°$ (*c*, 1% in chloroform). Found: C, 57·6; H, 5·6. $C_{25}H_{28}O_{12}$ requires C, 57·7; H, 5·5%.

Whereas the powder prepared from the urine contained no free 1:2-dihydronaphthalene-1:2-diol, this compound was present after treatment of the powder with β-glucuronidase. The diol thus liberated had the following properties: m.p. 127°; $[\alpha]_D^{25°} = +158°$ (*c*, 1% in ethanol), and it was identical with the dextrorotatory diol obtained by Booth & Boyland (1949) from the urine of rabbits dosed with naphthalene.

The evidence obtained suggests, therefore, that rabbits excrete a monoglucuronide of 1:2-dihydronaphthalene-1:2-diol after they have been dosed with naphthalene. The glucuronic acid of the conjugated compound appears to be attached to the 1-position of the naphthalene ring structure, for acid treatment of the glucuronide-containing powder resulted in the formation of 1-naphthylglucuronide.

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Stereochemical Aspects of the Metabolism of Ethylbenzene. By J. N. Smith, R. H. Smithies and R. T. Williams. (*Department of Biochemistry, St Mary's Hospital Medical School, London, W. 2*)

Ethylbenzene is hydroxylated in the body at the β-carbon of the side chain (Neubauer, 1901; Thierfelder & Daiber, 1923) to form phenylmethylcarbinol. Acetophenone is reduced *in vivo* to phenylmethylcarbinol which is excreted as a glucuronide (Thierfelder & Daiber, 1923). A possible mechanism for this hydroxylation is

$$PhCH_2CH_3 \rightarrow PhCH{:}CH_2 \rightarrow PhCOCH_3 \rightarrow PhCHOHCH_3.$$

The glucuronic acid conjugations of equivalent doses of the above compounds in rabbits were measured and found to be: ethylbenzene, 32; styrene, 16; acetophenone, 47; (±)-phenylmethylcarbinol, 50% of the dose. The conjugation of β-phenylethanol, $PhCH_2CH_2OH$, was low (7%), the main metabolites being phenylacetic acid conjugates.

The low value for the conjugation of styrene suggests that it is not a major intermediate in ethylbenzene hydroxylation.

Acetophenone also seems to be ruled out as a major intermediate because the glucuronide isolated from the urine of rabbits fed with acetophenone was entirely that of (−)-phenylmethylcarbinol (isolated as *triacetyl* β-(−)-*phenylmethylcarbinyl*-D-*glucuronide methyl ester*, m.p. 113° and $[\alpha]_D^{20°} - 83°$ in $CHCl_3$). The reduction of acetophenone was thus asymmetric. The triacetyl methyl ester from ethylbenzene urine had $[\alpha] - 50{\cdot}2°$, from which the (−)diastereoisomer (m.p. 113° and $[\alpha]_D - 83°$) was isolated by fractional crystallization and an impure (+)diastereoisomer (m.p. 130–136° and $[\alpha]_D - 10°$). The hydroxylation of ethylbenzene *in vivo* thus produced both isomers of phenylmethylcarbinol.

On feeding (±)-phenylmethylcarbinol, the isolated triacetyl methyl ester of the glucuronide had $[\alpha]_D - 50°$. Fractional crystallization yielded the pure (−)isomer ($[\alpha]_D - 83{\cdot}5°$) in good yield and a small amount of impure (+)isomer ($[\alpha]_D - 17{\cdot}8°$).

A possible mechanism of hydroxylation is abstraction of a hydrogen atom from the β-carbon by an enzyme and then addition of ·OH to the resulting free radical thus:

$$PhCH_2CH_3 \xrightarrow{-H\cdot} Ph\dot{C}HCH_3 \xrightarrow{+\cdot OH} (\pm)PhCHOHCH_3.$$

The oxidation of ethylbenzene to phenylmethylcarbinol and acetophenone by free radicals has been shown to occur in purely chemical systems (Emerson *et al.* 1948; Merz & Waters, 1949).

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Glucosulphatase Activity in Marine Molluscs. By K. S. Dodgson and B. Spencer. (*Physiology Institute, Newport Road, Cardiff*)

The existence in certain tropical marine molluscs of a sulphatase capable of liberating sulphuric acid from glucose-6-monosulphate has been reported by Soda (1936), who suggested the name glucosulphatase for the enzyme. It has not been established with certainty that molluscs common to British waters possess this enzyme, although Percival (1949) claims some indications of its presence in these organisms.

A number of herbivorous and carnivorous molluscs have been examined in this laboratory, but only the large periwinkle (*Littorina littorea*) possesses appreciable glucosulphatase activity. A crude enzyme concentrate has been obtained by preparing an acetone-dried powder of periwinkle viscera and subsequent fractionation of an aqueous homogenate of this powder with acetone. The final product shows considerable glucosulphatase activity as determined by the method of Dodgson & Spencer (1952); 1 g. of the powder liberating 48·8 mg. SO_4 from approximately 0·03 M-potassium glucose-6-sulphate in 0·5 M-acetate buffer, pH 5·8, in 1 hr. at 37·5°. The concentrate also possesses appreciable β-glucuronidase and aryl- and chondro-sulphatase activity.

Maximum enzyme activity towards potassium

glucose-6-sulphate occurs at pH 5·8 when the substrate concentration is approximately 0·01 M. Preliminary experiments indicate the optimum substrate concentration to be greater than 0·01 M, but this point has not been pursued further, since there are indications that the substrate is not homogeneous. When prepared by the method of Duff (1949), barium and potassium glucose-6-sulphates are hydrolysed by 0·1 M-hydrazine at pH 5·5 and at 37·5° to an extent of about 15 % in 36 hr., the degree of hydrolysis varying slightly from preparation to preparation. No further hydrolysis can be achieved either by prolonged incubation or by addition of more hydrazine. On the other hand, addition of more substrate results in a further hydrolysis of about 15 % of the extra material. These findings agree with those of Egami (1938) for barium glucose-6-sulphate prepared by the method of Soda (1933). Soda & Egami (1942) noted that this partial hydrolysis could be almost completely eliminated by preparing glucose-6-sulphate via a fractionally crystallized brucine salt, and concluded that sulphation of glucose by the normal procedures gave more than one monosulphated product.

The preparation of pure potassium glucose-6-sulphate by a more convenient method is being studied in this laboratory before continuing enzyme studies with this substrate.

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Composition and Cytology of *Nitrosomonas*. By T. Hofman and H. Lees. (*Department of Biological Chemistry, University of Aberdeen*)

Pure cultures of *Nitrosomonas* were grown on a medium consisting chiefly of calcium and magnesium carbonates, phosphate and ammonium sulphate (Lees, 1952).

For amino-acid and sugar analyses the insoluble inorganic salts were dissolved in 5 % acetic acid and the washed organisms were hydrolysed with 6N-hydrochloric acid and with sulphuric acid respectively. The hydrochloric acid hydrolysate was analysed for total nitrogen, amino nitrogen (Pope & Stevens, 1939) and free ammonia. Two-dimensional paper chromatograms (phenol/butanol-acetic acid) were run for the identification of the amino-acids present. The separation of the leucine isomers was achieved by running a one-dimensional paper chromatogram in *tert.*-amyl alcohol in the presence of diethylamine (Work, 1949). The method of Fowden (1951) with small modifications was used for the determination of the amino-acids. The following results in terms of amino nitrogen as percentage of total nitrogen were obtained: aspartic acid 5, glutamic acid 8, serine 2, glycine 8, threonine 4, alanine 8, tyrosine 1, leucine 3, isoleucine 4, valine 7, arginine 2, histidine 1, lysine 4, phenylalanine 4, methionine 2, proline 4, ammonia 8.

Cystine was present in small amounts but was not estimated.

The amino-acid pattern is very similar to that obtained from a hydrolysate of *C. diphtheriae* (Work, 1949) except that no diaminopimelic acid has been found. No unidentified spots were detected on the chromatograms.

One- and two-dimensional chromatograms of a sulphuric acid hydrolysate revealed the presence of four sugars which were chromatographically identical with galactose, ribose, rhamnose and xylose, the latter being present only in traces. No glucose was found. Chromatograms sprayed with *p*-anisidine hydrochloride (Hough, Jones & Wadman, 1950) revealed an additional spot with a strong bluish fluorescence in the ultraviolet light. Its identity has not so far been established.

Electronmicrographs—taken by Mr J. A. Gard, Chemistry Department—showed that the organisms which were freed of the insoluble inorganic salts by fractional centrifugation possess one 'nuclear body' in each cell. Some photographs gave evidence of further organization inside this body. The formation of zooglea was clearly observed.

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The Chromatography of Bile Pigments with Particular Reference to the Van den Bergh Reaction. By P. G. COLE and G. H. LATHE. (*Bernhard Baron Memorial Research Laboratories, Queen Charlotte's Maternity Hospital, London, W.* 6)

Previous workers have noted the tenacity with which adsorption columns retain bile pigments unless they have first been esterified. To avoid this, in view of the instability of bile pigments in the mildest chemical procedures, separation of bile pigments has been attempted on partition columns.

For a preliminary separation of pigments a reverse phase column of silicone-treated Kieselguhr (Howard & Martin, 1950), using the two phases from 25 % CCl_4, 25 % $CHCl_3$, 38 % MeOH, 6 % water and 6 % (v/v/v/v) pH 6 phosphate buffer (Cole, 1933) is satisfactory. With a ratio of mobile phase to stationary phase of 6, bilirubin moves as a discrete band with an R_F of 0·6. Under the same conditions human autopsy bile yields a group of pigments, most of which move more rapidly.

Pigments were extracted from jaundice sera by the addition of 0·18 vol. of saturated ammonium sulphate and 2·5 vol. of ethanol, followed by centrifuging. Extracts from sera of patients with obstructive jaundice showed two yellow bands, a prominent one faster than bilirubin, and a small one moving as slow as, or slower than, bilirubin. Extracts of sera from cases of haemolytic jaundice (of the newborn) showed similar bands, the slower being much more pronounced, and the polar one being greatly reduced, as compared with sera from cases of obstructive jaundice.

The polar band, moving faster than bilirubin, gives a direct van den Bergh reaction, while bilirubin (after passage through a column), and the slow-moving band from jaundice sera, react indirectly in the van den Bergh test.

The movement of the polar, direct-reacting band from sera from obstructive jaundice patients is too rapid on the above column for good resolution. When it is run on a reverse phase column of 50 % $CHCl_3$, 30 % MeOH, and 20 % H_2O (v/v/v), with a ratio of mobile phase to stationary phase of 3, the band splits into two pigments, both of which produce colour in the van den Bergh reaction. This has been confirmed on other columns, but an entirely satisfactory system has not yet been achieved.

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The Application of Counter-Current Methods to the Fractionation of Lipid Material from Brain. By P. G. COLE, G. H. LATHE and C. R. J. RUTHVEN. (*The Bernhard Baron Memorial Research Laboratories, Queen Charlotte's Maternity Hospital, London, W.* 6)

A solvent system (A) composed of CCl_4 62 %, MeOH 35 % and H_2O 3·15 % (v/v/v) was devised to minimize emulsion formation in the counter-current distribution of lipids. With this solvent system crude preparations of brain lecithin, cephalin, sphingomyelin and cerebroside, as well as crude brain lipid, were distributed through 49 plates (tubes) in the glass and polythene apparatus previously described (Lathe & Ruthven, 1951). In this apparatus the lower layer (non-polar in solvent A) was moved.

A comparison of the weight curves of distributions indicated that the cephalin fraction contained more polar material than the sphingomyelin and cerebroside, while lecithin showed peaks of both polar and intermediate polarity. The distribution of crude lipid from brain in system A gave rise to three groups of fractions, very polar (tubes 0–4), polar (tubes 5–17) and one of intermediate polarity (tubes 18–40).

For further separation of polar fractions, the polarity of solvent A was altered by the addition of chloroform and methylene chloride or alternatively the phase ratio (upper/lower) was changed from 1/1 to 1/4. For the fractions of intermediate polarity redistribution in a system modified from A by the addition of light petroleum led to separation of cholesterol from phospholipins.

The analysis of fractions for nitrogen, phosphorus, sugars, cholesterol, and the identification of bases by paper chromatography, showed that there was considerable overlapping of many components. Amino-acids, reducing material, and breakdown products were present in the most polar tubes (0–4) of the distribution of crude brain in system A. A lecithin fraction was probably included in these tubes. Phosphatidyl ethanolamine and phosphatidyl serine were spread through tubes 4–16. In the central region peaks were found as follows: sphingomyelin tube 21, lecithin tube 27, cerebroside tube 29 and cholesterol tube 35. A small amount of non-polar material was present in the position of neutral fat at tube 45.

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A New Brief Method of Estimating Net Protein Value. By A. E. BENDER and D. S. MILLER. (*The Crookes Laboratories, Ltd., Park Royal, London, N.W.* 10)

The conventional Thomas-Mitchell method of estimating the biological value (B.V.) of proteins is to measure the proportion of the absorbed N retained in the body. B.V. × digestibility gives the net protein value (N.P.V.).

Let N intake on test diet = I_f; N intake on non-protein (or 4·5 % egg protein) diet = I_K; total faecal N = F; metabolic faecal N = M; urinary N = U; endogenous urinary N = E; then, by definition,

$$\text{N.P.V.} = \frac{I_f-(F-M)-(U-E)}{I_f-(F-M)} \times \frac{I_f-(F-M)}{I_f}$$

$$= \frac{I_f-F+M-U+E}{I_f}.$$

Subtract I_K/I_f from both sides of the equation,

$$\text{N.P.V.} - \frac{I_K}{I_f} = \frac{(I_f-F-U)-(I_K-M-E)}{I_f}.$$

I_f-F-U is the gain in N by the animal fed a protein diet = final carcass N − initial carcass N = B_f-B. I_K-M-E is the change in N incurred by feeding non-protein diet (or 4·5 % egg protein) = B_K-B

$$\text{N.P.V.} - \frac{I_K}{I_f} = \frac{(B_f-B)-(B_K-B)}{I_f},$$

$$\text{N.P.V.} = \frac{B_f-B_K+I_K}{I_f}.$$

This implies the impossible requirement of a carcass N analysis on the same rat fed the non-protein and test diets. The carcass N on the non-protein diet may be estimated by the use of control animals which should be litter mates of equal weight to the test animals.

In practice four litters of eight rats are divided by litters into groups of four so that each group totals the same weight. One group is fed non-protein diet (or 4·5 % egg protein) and the remaining seven groups fed seven test proteins at the 10 % level for 10 days. The animals are killed and the body N determined by Kjeldahl digestion of the whole carcass. The analyses of the four rats in each group are combined and substituted in the equation above.

Seven estimations of casein made in three separate experiments gave 59, 60, 55, 56, 58, 60 and 57, i.e. 57·9 ± 0·7 (S.E.). Other results were: commercial egg albumen 82, whey proteins 86, commercial dog-fish meal 51, and extracted bran protein 57.

The values for casein were compared with that obtained by the slope method of Allison & Anderson (1945) feeding at 4, 8, 10, 12 % levels. This method gave a net protein value of 59. The rats fed at the 10 % level were used for the carcass analysis method and gave a value of 54.

Egg albumen and casein were evaluated by the full Thomas-Mitchell method according to the procedure of Henry, Kon & Watson (1937) which showed B.V. of albumen 81·8 ± 2·62, digestibility 97·9 ± 0·5 %, casein B.V. 66·5 ± 1·76, digestibility 98·9 ± 0·3 %. The carcass analysis assay carried out simultaneously gave N.P.V. 82 and 57 ± 1·5 (triplicate assay) respectively.

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Constancy of the N/H_2O Ratio of the Rat and its Use in the Determination of the Net Protein Value. By A. E. BENDER and D. S. MILLER. (*The Crookes Laboratories, Ltd., Park Royal, London, N.W.* 10)

The Thomas-Mitchell method (Mitchell, 1923–4) of estimating biological value involves large numbers of N analyses of both urine and faeces. The development described in the previous paper reduces this to a single analysis on the whole body of the animal. It is shown here that the estimation can be replaced by a determination of the water content of the animal.

It was shown by Moulton (1923) that body constituents, including the N and water content, are a constant proportion of the body when measured on a fat-free basis. It follows that the ratio of N/H_2O is a constant and therefore the N content of the body can be derived from the water content.

One hundred and forty-seven rats aged 33–57 days were analysed for total body N and water, and the correlation between this N/H_2O ratio and age was found to be expressed by $y = 2{\cdot}92 + 0{\cdot}024x$, where y is N/H_2O % and x the age in days. The correlation coefficient was 0·42 ($P < 0{\cdot}001$), indicating a substantially linear relation over this short age range. These rats had been fed a wide range of diets varying from non-protein for 10 days to 20 %

protein for 3 weeks without affecting the N/H_2O ratio. The ratio for a 38-day-old rat is 3·8, which compares with a value of 3·92–3·97 calculated from the data of Light, Smith, Smith & Anderson (1934) for six rats. We do not yet know whether this ratio measured on our black and white hooded colony is applicable to other colonies.

The measurement of the net protein value is carried out as described in the previous paper but with the body N analyses replaced by a dry weight determination. After the 10-day feeding period the animals are killed with chloroform and weighed. Incisions are made into the skull and thoracic and body cavities and the carcasses dried to constant weight at 105° (24 hr.). The water content is converted to N by multiplying by the appropriate ratio, taking account of the age of the animal. The body N figures are substituted in the equation,

$$\text{net protein value} = \frac{B_f - B_K + I_K}{I_f}.$$

The following net protein values were obtained by this method, the values in brackets being those obtained by an actual N analysis of the rats. Extracted bran protein 58 (57), whey proteins 89 (86), commercial dogfish meal 55 (48), mixture FW 73 (72), casein determined seven times $55·1 \pm 1·7$ ($57·9 \pm 0·7$), diet M determined three times $58·6 \pm 2·3$ ($56·6 \pm 2·2$), egg albumen 83 (82).

This method would permit estimation of body N on the same subject both before and after feeding test diets, i.e. N balances, without sacrificing the animal, simply by measuring the body water by one of the accepted methods (isotope dilution, urea or antipyrine) and might be applicable to man.

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The Incorporation of Labelled Phosphate into the Lipids of a Guinea-pig Brain Homogenate.

By R. M. C. Dawson (Betty Brookes Research Fellow). (*Department of Biochemistry, University of Oxford*)

Cell-free homogenates of guinea-pig brain have been incubated in Ringer solutions containing labelled phosphate (^{32}P) and measurements made of the uptake of radioactivity by the lipids. A simple technique was evolved for the extraction and purification of the phospholipins. A chloroform/methanol extract of these was equilibrated with 2 vol. of N-HCL or 0·25 M-$MgCl_2$ whereby protein matter and contaminating acid-soluble P passed into the aqueous phase. By this means the zero-time uptake of labelled phosphate was reduced to a few per cent of the full enzymic incorporation.

For maximum uptake of ^{32}P the system required the presence of Mg^{++}, adenylic acid, cytochrome *c*, oxidizable substrate and O_2. The optimum pH was near 6·5. Nearly all the uptake occurred in the first hour of an incubation at 37°, the subsequent incorporation being very slow. Anaerobic glycolysis supported the uptake of phosphate only to a limited extent. The presence of F^- greatly activated the incorporation when the homogenate was respiring with a substrate of pyruvate plus fumarate. The uptake of phosphate could be uncoupled from the respiration of the homogenate by the presence of 2:4-dinitrophenol, azide, methylene blue, arsenate, gramicidin, SO_4^{--}, Ca^{++}, and with hypertonic Ringer. The incorporation was also inhibited by glucose, creatine, inorganic P, and in some conditions by adenosinetriphosphate. The addition of a number of coenzymes and possible elementary precursors of the phospholipins had no effect on the uptake of labelled phosphate. These results suggest that the uptake of phosphate into the phospholipin fraction of brain homogenates is dependent upon oxidative phosphorylation: this being rate limiting for the incorporation.

In further experiments the brain phospholipins have been fractionated using alcoholic precipitation, MgO adsorption, and saponification. The results show that the uptake is localized in the 'kephalin' fraction with practically no synthesis of labelled lecithins or sphingomyelins. The uptake into phosphatidyl ethanolamine has been measured by a new method based on catalytic hydrolysis with $HgCl_2$, and the results confirm earlier ones which showed that the synthesis was negligible in brain homogenates (Norman & Dawson, 1952). This indicates that the incorporation is confined solely to a fraction containing phosphatidyl serine, diphosphoinositide and possibly phosphatidic acids.

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Two Selective Inhibitors of Cholinesterase. By L. AUSTIN and W. K. BERRY. (*Chemical Defence Experimental Establishment, Porton, Wilts*)

Two new inhibitors have been described which, in view of their reported high selectivity towards 'true' and 'pseudo' cholinesterases, appeared to be useful tools for examination of some ChE's which do not fit the conventional classification (Ord & Thompson, 1951; Earl & Thompson, 1952; Davies, Risley & Rutland, 1952). They are, bis(bis-*iso*propylamino)-phosphonous anhydride—*iso*OMPA—reported by Aldridge (1952) to be 10000 times more active against the 'pseudo' ChE of horse serum than against the 'true' ChE of horse erythrocytes: and 1–5-bis(4-allyl dimethylammonium phenyl)pentan-3-one dibromide—284C51—found by Fulton (1952) to inhibit 'true' ChE but not 'pseudo'.

Using the enzymes of human blood, we have found that *iso*OMPA irreversibly inactivates the plasma 'pseudo' ChE. It attacks the active centre, and the kinetics of inhibition are bimolecular. 284C51 is a competitive reversible inhibitor of the erythrocyte 'true' ChE.

The selectivity of the inhibitors (expressed as the ratio I_{50} cell ChE:I_{50} plasma ChE) has been measured using the blood of four mammalian species as sources of 'true' (erythrocyte) and 'pseudo' (plasma) ChE. Substrate studies indicated the absence of 'pseudo' ChE from the erythrocytes, and vice versa. The selectivity of each inhibitor was generally high, but species variations exist. It appears possible that appropriate concentrations of both inhibitors should inhibit one type of ChE 95% while affecting the other by not more than 5%.

The finding by Earl & Thompson (1952) that fowl plasma contains one ChE, as sensitive to inhibitors as 'pseudo' ChE but capable also of hydrolysing acetyl-β-methyl choline, has been confirmed.

We are indebted to Dr Copp of the Wellcome Research Laboratories for a supply of 284C51. Acknowledgement is made to the Chief Scientist, Ministry of Supply, for permission to communicate these results.

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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **312th Meeting** *of the Biochemical Society was held in the London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C. 1, on Saturday, 15 November* 1952. *The scientific business of the meeting commenced at* 11.0 *a.m. and was in the form of a Symposium on 'Immunochemistry'.*

COMMUNICATIONS

Immunity Reactions and the Structure of Proteins. By J. R. MARRACK. (*Department of Chemical Pathology, London Hospital Medical College, E.* 1)

Antigens are attracted to antibodies by short-range non-specific forces. The highly specific affinity must depend on the close fit of parts of the antigen molecule (determinants) to parts of the antibody molecule (receptors). Three problems arise: (1) What is the structure of the receptors of antibodies? (2) What are the determinant groups of native proteins? (3) Why do compounds of antigens and antibodies form precipitates?

(1) Antibodies that combine specifically with certain groups can be made by immunizing with proteins coupled to the groups. The most effective groups are ionized. It appears that the receptors for such ionized groups consist of (*a*) an oppositely charged group in a cavity which the determinant will not fit unless of suitable shape and size; and (*b*) a relatively large non-specific part to which the determinant is held by Van der Waals forces.

(2) The determinants of native proteins must be formed of sets of side chains of amino-acids arranged in a specific pattern. The variety of orientations of side chains round the helix of Pauling, Corey & Branson (1951) would explain the great range of specific differences between proteins.

(3) The possibility that there may not be room on an antigen molecule for the number of antibody molecules demanded by the lattice theory and the theory of Goldberg & Williams (1952) are discussed.

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Antigen-Antibody Interaction using Enzymes as Antigens. By B. CINADER.* (*The Lister Institute of Preventive Medicine, London, S.W.* 1)

Enzymes are proteins and therefore act as antigens. Antibodies have been prepared against a large number of enzymes, and it has been observed that some antibodies inhibit the homologous enzyme completely whilst others seem to inhibit only partially or not at all. The relevant literature will be briefly summarized, and possible causes of the failure of some antibodies to inhibit will be discussed from the point of view of the nature of the inhibitory action of antibodies and the sites on the antigen against which antibodies are formed.

Using enzymes as antigens it is possible to investigate immunological systems analogous to those involving toxins and antitoxins which so far have been studied by animal experiments. Model systems will be described in which the breakdown of substrate molecules replaces the death of test animals as indicator.

Roux & Kraus pointed out the difference between the action of an antibody when it is mixed with the toxin before injection (Ehrlich test) and when it is injected into the animal simultaneously with the toxin but at a different site. In the latter situation there is a competition for toxin between the affected tissue and antitoxin. The competition of substrate and antibody for the toxin can be investigated by comparing a system in which enzyme and antibody are incubated before addition of the substrate with a system in which the enzyme is added to a mixture of substrate and antibody. This will be demonstrated by an account of experiments on the inhibition of lecithinase (α-toxin of *Clostridium welchii*) by its equine antibody, and the nature of the inhibition of this enzyme by its antibody will be discussed.

* Beit Memorial Fellow.

The Reactions of Toxins with Antitoxins. By W. E. VAN HEYNINGEN. (*Sir William Dunn School of Pathology, Oxford University*)

Unlike enzymes, toxins cannot be assayed accurately by measuring their activity because in most cases their modes of action and their substrates are not known. However, they can be assayed with reasonable accuracy and convenience by titrating them against their antitoxins. The end point of the titration is either seen indirectly in the action of excess toxin on whole mice, red blood cells, guinea pig skin, etc., or directly in toxin-antitoxin flocculation. These methods of assay measure the concentration of toxin as antigen and bear little or no relation to its biological activity.

The activity of toxins and enzymes can be lost spontaneously or destroyed by treatment with a variety of reagents in such a way that the antigenic properties are retained (toxoiding). The mechanism of toxoiding is not understood, probably because there are many different mechanisms.

Although the activities of almost all toxins and toxic enzymes are inhibited by homologous antibodies *in vivo* there are many enzymes whose actions *in vitro* are not inhibited by antibody. During *in vivo* neutralization other factors besides antibody play a part. Whether an enzyme is neutralized by antibody *in vitro* is a matter of chance, because catalytic and antibody-combining regions on proteins are not necessarily the same.

The Use of Isotopes in Immunology. By G. E. FRANCIS. (*Department of Biochemistry and Chemistry, St Bartholomew's Hospital Medical College, London*)

Isotopes are being increasingly used by immunologists, especially as labels for proteins in a variety of investigations in the field of immunochemistry.

The advantages and disadvantages of the isotopic labelling technique in this connexion will be discussed in some detail.

The use of antigens and antibodies labelled with radioactive isotopes enables quantitative studies of the antigen-antibody reaction to be carried out under conditions which would be very difficult, if not impossible, with the more classical methods. Analyses of a variety of protein-antiprotein specific precipitates have been carried out by several workers, using ^{131}I, ^{35}S and ^{32}P as labels. Investigations have also been carried out on antibodies to phospholipins and on the reaction between haptens and antibodies.

Rabbit antisera to rat and mouse lung, liver and kidney, labelled with ^{131}I and injected into rats and mice, have been shown to deposit selectively in the glomerular tissue of the kidney, and important deductions have been made as to the relationship of this phenomenon with the causes of glomerulonephritis.

Measurement of the disappearance rate of intravenously injected labelled antigens has been used as a test of immune response in rabbits, and as a means of investigating the mode of action of antibodies *in vivo*. Attempts have also been made to trace any tissue localization of these injected antigens in order to obtain more information as to the site of antibody formation.

Experiments on the biosynthesis of labelled antibodies have also produced some information on the mode of formation of antibodies.

Antigens and Antibodies in Hypersensitivity. By P. G. H. GELL. (*Department of Experimental Pathology, University of Birmingham*)

A sharp distinction is usually drawn between two types of hypersensitivity reactions, (i) those of an 'immediate' or histamine-like type, and (ii) those of a delayed, tuberculin type. Sensitivity to simple chemicals offers a convenient approach for the biochemist in that he can start with crystalline, low molecular weight, compounds. In experiments in guinea pigs, it has been possible to show the co-existence and independence of both types of reaction pattern. Hence the basic distinction is in the 'immune' reaction of the body, rather than in the antigen.

There are, however, a number of unsolved problems relating to natural and artificial antigens. For example, the exact nature of the reactions which chemical sensitizers undergo in the cell, the fractional purification of 'allergens' such as dust and horse dander, the course of the enzymic reactions by which contact with allergen provokes the release of histamine, are all problems demanding a biochemical approach; current work on these will be discussed.

The antibody in anaphylaxis-asthma type sensitivity, though present in the serum and sometimes

identical with precipitin, is often of non-precipitating and more obscure nature; the 'antibody' in tuberculin-type sensitivity, though not present in the serum, is transferable by means of cells; interesting work along both these lines is proceeding. The pathologist can try to correlate histological changes with biochemical events, and examples of such an approach will be discussed. The use of radioactive markers is here of great potential value.

The Role of Carbohydrates in Immunochemistry. By M. STACEY. (*Department of Chemistry, University of Birmingham*)

A brief review will be made of the structure and serology of pneumococcal type-specific capsular polysaccharides and of the group-specific substance. Recent structural investigations on the type II specific polysaccharide will be reported, and serologically cross-reacting bacterial dextrans will be commented upon.

The structure and significance of dextrans and levans will be outlined. Present-day knowledge on specific polysaccharides from *Mycobacterium tuberculosis*, on carbohydrate heterophile antigens from the Salmonellae and on blood-group factors will be summarized.

The Relation of Complement to the Nitrogen Bound by Antigen-Antibody Precipitates. By R. G. S. JOHNS. (*Hale Clinical Laboratory, The London Hospital, E.* 1)

Complement has been known since 1895, but until 1941 only as a disembodied collection of lytic properties. In that year Heidelberger and his colleagues (see, for example, Heidelberger & Mayer, 1948) first demonstrated that the mass (in terms of nitrogen) of an antigen-antibody precipitate formed in the presence of active complement is greater than in its absence. The 'complementary nitrogen' bound by a precipitate has not been unequivocally identified with haemolytic complement, and the work of Heidelberger has been confined to the equivalence zone of the antigen-antibody reaction.

The present communication will report findings on the complementary nitrogen bound by antigen-antibody precipitates in the regions of antigen and antibody excess, using human and horse sera as complement sources, and also with bovine serum as a source of conglutinin, a material which may be described as being 'complementary to complement'.

The results obtained are difficult to interpret and further work is indicated, but they do not accord with Heidelberger's theory of complement fixation. It would appear that the difference between human and horse complements is more fundamental in character than is implied by the present view of a deficiency of second component in horse complement.

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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **313th Meeting** *of the Biochemical Society was held in the Department of Biochemistry and Chemistry, The Medical College of St Bartholomew's Hospital, Charterhouse Square, London, E.C.* 1, *on Friday, December* 19*th*, 1952, *at* 2.0 *p.m., when the following papers were read:*

COMMUNICATIONS

The Hydrolysis of Choline Esters by Tissues of the Ruminant. By D. R. Davies, J. E. Risley and J. P. Rutland. (*Chemical Defence Experimental Establishment, Porton, Wilts*)

The observation that ruminant tissues do not hydrolyse benzoylcholine as fast as do rat tissues suggested that the distribution of the so-called 'pseudo' cholinesterase might be markedly different in these two species, and indicated the necessity for a closer study of this difference if the significance of this enzyme in acetylcholine metabolism was to be appreciated more fully.

We have examined substrate specificity patterns using sheep and rat tissues. Five esters of choline have been examined, acetyl- (ACh), benzoyl- (BCh), acetyl-β-methyl- (MCh), butyryl- (BuCh) and propionyl-choline (PrCh). The results may be summarized as follows:

(1) The activity of sheep tissues against ACh is very much lower than that of rat tissues.

(2) Few sheep tissues except the kidney hydrolyse BCh to any great extent.

(3) Rat tissues present a far more constant pattern of substrate specificity than sheep. Thus in the rat the PrCh/BuCh activity ratio varies from only 1·4 to 2·0. In the sheep the variation is from 0·4 to 3·0.

(4) The BuCh/BCh and PrCh/BCh values also are irregular in the ruminant. They are much more uniform in the rat.

(5) Rat kidney is an exception. These ratios are much higher than in any other rat tissue.

(6) In most sheep tissues BuCh is hydrolysed more rapidly than PrCh.

The blood, both erythrocytes and plasma, has also been examined, from humans, sheep and rats, in the same manner. The substrate patterns vary quite markedly.

These preliminary results suggest either the existence of a number of esterases in ruminant tissue or that the environment in which an enzyme is found may well modify its behaviour. These results also emphasize the danger of examining ChE variation in tissues using only one criterion of activity.

Acknowledgement is made to the Chief Scientist, Ministry of Supply, for permission to communicate these results.

An Activator of Prostatic Acid Phosphatase. By Grace M. Jeffree. (*University of Bristol, Medical Research Laboratory*)

Experiments with prostatic acid phosphatase suggest the presence in serum and other biological fluids of a substance which is necessary for full activity of the enzyme.

In the estimation of acid phosphatase values in seminal fluid, it is usual to work with high dilutions in physiological saline (Gutman & Gutman, 1941; Watkinson, Delory, King & Haddow, 1944). It was found that such preparations often give markedly lower values than similar dilutions in serum or serum derivatives; and the same was found for dilutions of saline extracts of human prostate. In some instances, where specimens of seminal fluid had been kept for a period in the refrigerator, it was found that high dilutions in saline showed no acid phosphatase activity, whereas similar dilutions in serum derivatives still showed full activity.

The activating substance was found to be present in excess in serum, 1/10 dilutions of serum being as effective as serum itself in maintaining phosphatase activity of values up to about 30 units per 100 ml., using the Gutman & Gutman (1940) modification of the method of King & Armstrong (1934). The substance is present in a variety of natural fluids, being found in normal female urine, in cerebrospinal fluid, in orange juice and yeast extract. It is thermostable, and is still present in a filtered watery extract of serum which has been coagulated by heating on a boiling-water bath. It is resistant to treatment by normal HCl, H_2SO_4, NaOH and KOH, but is destroyed by concentrated H_2SO_4 and by incineration. It is not replaced by Na, K, Ca or Mg ions.

The substance would appear to be a comparatively small organic molecule. It is not replaced by ascorbic acid, though, in agreement with Moog (1944), this was found to have some activating effect on the enzyme. It does not appear to be

identical with the substance described by Kutscher & Sieg (1950) as a coenzyme of acid muscle phosphatase and alkaline kidney phosphatase, and provisionally identified by them as choline pyrophosphate, since this is completely destroyed by 1 hour's boiling of the aqueous solution or by 30 minutes' treatment with 0·1N-NaOH at room temperature and its solubility in organic solvents is considerably greater than was found for the activator of prostatic phosphatase.

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Ali-esterase Inhibition by Tri-*ortho*-cresyl Phosphate. By B. Mendel and D. K. Myers. (*Pharmaco-Therapeutic Laboratory, University of Amsterdam, Amsterdam, Holland*)

Hottinger & Bloch (1943) found that tri-*ortho*-cresyl phosphate (TOCP) inhibits cholinesterases; the preferential action of TOCP on pseudo-cholinesterase *in vivo* was described by Mendel & Rudney (1944) and studied *in vitro* by Earl & Thompson (1952). Much smaller amounts of TOCP than are required for the inhibition of the cholinesterases exert—*in vitro* and *in vivo*—an inhibitory action on the serum and liver ali-esterases (tributyrinases) of rats (Mendel & Mortimer, 1944).

The hydrolysis of tributyrin by 2% rat serum is about 50% inhibited by 300 μl. TOCP/l. after incubation at 37·5° for 30 min. and by 0·6 μl. TOCP/l. after incubation for 20 hr. The slow reaction between ali-esterase and TOCP is unaffected by plasma proteins and appears to be irreversible. 20 hr. after the intramuscular injection of about 50 μl. TOCP/kg. into rats, the serum tributyrinase is almost completely inhibited, remains inhibited for 2–3 days and subsequently returns to normal very slowly; the pseudo-cholinesterase activity is not affected appreciably.

It was observed that some preparations of TOCP may appear to be a few hundred times more active than others against ali-esterase after 30 min. incubation *in vitro*; the differences after 20 hr. incubation are not so great. A suggestion that these discrepancies in initial inhibitory activity might be due to a reactive impurity in the TOCP preparation (Aldridge, 1952) has been confirmed. However, there is little difference between the pure and impure TOCP preparations in their effects on the esterases of the rat *in vivo*; a pure preparation may be slightly more active *in vivo*.

The order of sensitivity of the esterases to inhibition by TOCP and other phosphate esters generally is: serum tributyrinase > pseudo-cholinesterase > true cholinesterase ≧ brain tributyrinase. Many other ali-esterases show intermediate degrees of sensitivity to inhibition by TOCP; some esterases may be completely insensitive not only to TOCP but also to other organo-phosphorus inhibitors. The tributyrinase of rat erythrocytes resembles that of rat brain in its low sensitivity to TOCP, whereas the tributyrinase of dog erythrocytes is very sensitive to inhibition by TOCP *in vivo*. After a single feeding of 20 μl. TOCP/kg., this latter esterase is completely inhibited and only after 2–3 months will it have returned to its normal level (Mendel & Mortimer, 1944).

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Prevention of the Androgenic Effects of Testosterone Propionate by Ali-esterase Inhibition. By D. K. MYERS and S. P. SIMONS. (*Pharmaco-Therapeutic Laboratory, University of Amsterdam, Amsterdam, Holland*)

According to Mendel & Mortimer (1944), tri-*ortho*-cresyl phosphate (TOCP) is a selective irreversible inhibitor of the ali-esterase (tributyrinase) in rat serum *in vitro* and *in vivo*; it can also be used to inhibit the tributyrinases in rat liver (Mendel & Mortimer, 1944; Myers & Mendel, 1953), intestinal mucosa, adrenal gland, testis, seminal vesicle and prostate gland. Moreover, TOCP has very little toxicity for rats (Smith, Engel & Stohlman, 1932).

This inhibitory action of TOCP has therefore been utilized to study the possible functions of ali-esterases in the testis and accessory male glands. The low ali-esterase activity of the testis and seminal vesicle of immature rats is fairly resistant to inhibition by TOCP; however, 7–10 days before puberty, a new TOCP-sensitive esterase appears and the total esterase activity rises to several times the previous level (cf. Huggins & Moulton, 1948). This suggested that these ali-esterases might be concerned with the hydrolysis and possibly synthesis of testosterone esters (cf. Dingemanse, Freud & Polak, 1948).

To test this hypothesis, 3-week-old rats were injected with 200–500 μg. testosterone or 50 μg. testosterone propionate daily for 12 days, with and without additional subcutaneous injections of TOCP at total dose levels of 15 or 150 μl. Inhibition of the esterase in the accessory glands by TOCP strongly repressed the stimulation of the growth of seminal vesicle and prostate gland produced by testosterone propionate, but had little effect on the stimulation of growth produced by testosterone.

Histologically, the TOCP injections resulted in decreased mitosis in the seminal vesicles, a slight accumulation of sudanophilic lipid in the testis and marked lipid deposition in the adrenal cortex. Prolonged administration of large doses of TOCP for fourteen weeks caused testicular degeneration, grossly similar to that observed in vitamin E deficiency (cf. Draper, James & Johnson, 1952). However, the results strongly suggest that the effects of TOCP on the growth of the accessory glands in a short term experiment are due to ali-esterase inhibition rather than to vitamin E deficiency. Thus testosterone propionate does not seem to exert its androgenic effects before hydrolysis has taken place.

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Ionophoresis in Living Tissue. By E. J. HARRIS. (*Biophysics Department, University College, London*)

A small area of a tissue specimen can be made radioactive by the application of a solution containing radioactive tracer. If an electric field be applied across the specimen any charged tracer ions will tend to move (cf. Hodgkin & Keynes, 1951). The position of the tracer can be found by using a Geiger counter mounted behind a slit. The mobility can be calculated from observations of rate of movement and potential gradient.

Experiments are being made on sartorius muscles of frogs. At present it appears that at least a proportion of both sodium and potassium moves with a mobility of about 80 % of the value for the respective ion in free solution. Phosphate is peculiar in that a large proportion remains immobile for some hours. Movement of the remainder corresponds to a mobility less than that in solution, by the same ratio as found for Na and K ions. The immobilization of some phosphate seems to be in accord with previous observations (e.g. Causey & Harris, 1951) that a strong adsorption of this ion on to muscle takes place.

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Loss of Phosphate from Resting Frog Muscle. By E. J. HARRIS. (*Biophysics Department, University College London*)

The resting metabolism of isolated muscle might reasonably lead to the liberation of phosphate ions into the surrounding solution. The quantity of phosphate liberated has been measured by analysis and it proves to be very small indeed, between 0 and 6 μg. of phosphate P appearing from either sartorius or gastrocnemii in a period of several hours. The quantity is slightly higher initially, probably because of autolysis of damaged tissue, it then remains low for as long as 16 hr., but finally the cells die and lose phosphate, potassium, and their excitability.

In order to show that the small net transfer of phosphate to the solution was not a consequence of there being a very efficient re-incorporation of phosphate into the tissue use was made of ^{32}P labelled phosphate in the solution. From a solution having 1·4 μg. of phosphate P per ml. the transfer to the muscle was about 2 μg. in periods of either 3 or 16 hr.

It therefore seems valid to conclude that resting frog muscle does not lose appreciable phosphate to the surrounding solution.

Electrically Stimulated Cerebral Tissues in the Study of Drugs Acting on the Central Nervous System. By H. MCILWAIN. (*Biochemical Laboratories, Institute of Psychiatry (British Postgraduate Medical Federation, University of London), Maudsley Hospital, S.E. 5*)

Many drugs acting on the brain, including some which affect its carbohydrate metabolism and labile phosphate content *in vivo*, have no action on comparable characteristics in separated cerebral tissues when examined at concentrations approaching those acting *in vivo*. Several have now been examined on such characteristics in cerebral tissues whose metabolism has been stimulated by various means, especially electrically by applied impulses of various time-voltage relationships and spaced in different ways.

A rich variety of types of effect has been found. General depressants including barbiturates decreased respiration and glycolysis of mammalian cerebral cortex stimulated by several sorts of electrical impulses and by K^+ salts, in concentrations with little if any effect on the unstimulated tissue. Atropine (and cocaine) also had this effect on tissues stimulated electrically but not on those stimulated by K^+ salts. Anticonvulsants (bromides, 3:5:5-trimethyloxazolidine-2:4-dione, 5:5-diphenylhydantoin) differed from aliphatic barbiturates or urethane (but not from phenobarbitone) in having this action on tissues stimulated only by certain types of electrical impulse (Forda & McIlwain, unpublished). No effects have been found with eserine, acetylcholine, tubocurarine, nor with sulphonamides which inhibit carbonic anhydrase; but ergotoxine blocked the electrically induced respiration and glycolysis at 5×10^{-5} M. It is to be emphasized that the agents mentioned have negligible effects on the corresponding processes in unstimulated cerebral tissues at the concentrations used.

When in the separated stimulated tissue several metabolic characteristics associated with increased cerebral activity are changed together by an added agent, the agent probably affects actions which are akin to those following electrical stimulation of the intact brain and of other excitable tissues. Regarding this as involving a depolarization localized at any one time but transmitted, depolarization under the conditions studied is probably much more general; but by varying the time, voltage, and distance relationships of the applied impulses and comparing the effects of drugs with those on tissues stimulated by other agents, the action of drugs may be ascribed to changes in threshold, recovery time or spread of action and compared with such characteristics observed electrically in the brain and in other excitable tissues. Such considerations make it understandable that the electrically stimulated tissue can be a sensitive and flexible indicator of many agents affecting the central nervous system.

Determination of Radio-penicillin Uptake by Electrodialysis. By A. V. Few, P. D. Cooper and D. Rowley. (*The Wright-Fleming Institute of Microbiology, Paddington, London, W. 2*)

In previous experiments on the uptake of radio-penicillin by intact bacteria the excess penicillin has been removed by centrifuge washing, any radio-activity left on the bacteria being proportional to penicillin uptake (Rowley, Cooper, Roberts & Lester Smith, 1950). To detect the reaction between penicillin and non-dialysable substances an electro-dialysis cell was constructed of Perspex, across which a variable d.c. potential could be applied. Distilled water was fed by gravity through the platinum electrode end-compartments which were separated by cellophan membranes from a centre compartment containing 2·25 ml. of the substance to be dialysed.

The method had one main disadvantage. The pH of staphylococcal suspensions and egg albumin solutions rapidly fell to 4 units and rose very slowly. At this pH penicillin decomposition is no longer negligible over the time of experiment, and in fact the bacterial uptake of ^{35}S from penicillin by electrodialysis was high and resembled the uptake obtained from partially acid-inactivated radio-penicillin by the centrifuge washing method. Decomposition was therefore minimized by cooling in ice (the internal temperature, measured by an inserted thermocouple, was then always below 2°) and by removing penicillin more rapidly. Resin treatment of cellophan (Gilbert & Swallow, 1950), although little affecting the pH changes of staphylococcal suspensions, allowed faster dialysis of penicillin ions. The maximum current which could be passed (5 mA.), and hence the maximum rate of electrolyte removal, was limited by the heating effects due to the instrument's conductivity. Accordingly, before entering the instrument, the distilled water was passed through a column of Bio-deminrolit FF ion-exchange resin (Permutit Co.) to reduce the ionic content, when greater potentials could be applied initially. Samples to be dialysed were also as salt-free as possible.

The characteristic uptake curve of penicillin on a sensitive *Staphylococcus* then appeared the same by electrodialysis as by centrifuge washing, and this similarity was taken to indicate that, with the described precautions, the electrodialysis method was suitable to measure the uptake of penicillin on soluble large molecules.

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Effect of Light on Vitamin B_{12}. By F. Wokes, Nora Baxter, Janet Horsford and Barbara Preston. (*The Ovaltine Research Laboratories, King's Langley, Herts*)

Boxer & Rickards's (1951) method for the determination of vitamin B_{12} is based on liberation of cyanide by photochemical action. The product (from a solution in 0·1 N-H_2SO_4) was spectroscopically identical with sulphato-cobalamin (Kaczka, Wolf, Kuehl & Folkers, 1951). Since this product was reconverted to cyanocobalamin by treatment with excess cyanide, it was assumed that the only change in the molecule was the replacement of the cyano group by a sulphate group in the coordination complex of the cobalt atom.

A spectrophotometric study has been made of the action of light above 300 mμ. on cyanocobalamin in aqueous or acid solution (dilute HCl or H_2SO_4 added to bring pH below 4). The initial absorption maxima at 278, 361 and 548 mμ. were gradually replaced by maxima at 275, 350 and approx. 525 mμ. respectively. The absorption curves representing the changes in the 361 mμ. peak all passed through an isosbestic point at 355 mμ. supporting the assumption of a simple replacement in the molecule. In cyanocobalamin $E_{355\ m\mu.} = E_{366\ m\mu.}$ and in the photochemical product $E_{355\ m\mu.} = E_{345\ m\mu.}$ according to our present data. From these data have been calculated the proportions of the two substances in known mixtures, by the method of Morton & Stubbs (1946) and by the method of Daglish (1951). The results were in reasonable agreement with theory, minimizing the errors due to irrelevant absorption in cyanocobalamin solutions which have been exposed to light (Heathcote, 1952).

Application of these corrections showed that in Boxer & Rickards's (1951) method most of the cyanocobalamin is converted to the photochemical product within the first few minutes, but the cyanide thus liberated is carried over much more slowly into the NaOH traps. Hence the period of illumination can be much shorter than the period of aeration.

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The Effect of Anionic Detergents and Related Compounds on some Enzymes. By E. D. Wills. (*Department of Biochemistry and Chemistry, The Medical College of St Bartholomew's Hospital, London, E.C.* 1)

A study has been made of the effect of the detergent sodium dodecyl sulphate (SDS) on a variety of enzymes and the results have been compared with those obtained using several aromatic sulphonic acids including suramin (Wills & Wormall, 1950).

0·001M-SDS completely inhibited urease at pH 5·0, but inhibition fell off sharply with increasing alkalinity to zero at pH 5·4. Inhibition of urease was considerably less in weaker solutions of SDS, and although at a concentration of 0·002M reversible with pH it was irreversible at 0·001M. These results, when compared with those obtained using aromatic sulphonic acids may be interpreted as indicating that inhibition by the colloidal micelle of SDS is considerably more powerful than that of the dissociated ion.

Many other enzymes, e.g. invertase, peroxidase and the malt amylases were also found to be inhibited by SDS but, as in the case of urease, only if the pH was adjusted to a suitable acid value.

A comparison has been made of the effect of sodium taurocholate and several detergents on pancreatic lipase in order to ascertain if the bile salt could be replaced by these synthetic products.

SDS was found to belong to that class of substances which inhibit succinic dehydrogenase to a less extent than the complete succinic oxidase system and have no effect on cytochrome oxidase (cf. Slater, 1949). This effect is considered to be due to the combination of SDS with the methylene blue used in succinic dehydrogenase estimations.

In contrast to its effect on most enzymes SDS was found to accelerate the hydrolysis of some proteins by trypsin, an effect which may be explained by its powerful denaturing action on the protein substrates.

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The Fate of Intravenously Injected Proteins in Immune and Normal Rabbits. By G. E. Francis, J. D. Hawkins and A. Wormall. (*Department of Biochemistry and Chemistry, The Medical College of St Bartholomew's Hospital, London, E.C.* 1)

When small amounts of ^{131}I-labelled horse serum γ-globulins are injected into rabbits which have previously been immunized against horse γ-globulin, a considerable part of the injected antigen disappears from the blood stream in the course of a few minutes. Most rabbits were injected at the rate of 1 mg. N/kg. body weight and in these animals 30–80 % of the injected protein disappeared from the blood in 1 hr. When smaller injections were given (0·33 mg. N/kg.) practically all the antigen disappeared in the same time. Graphical analysis shows that the elimination rate in the first 2 hr. after injection is composed of two simultaneous exponential processes. One with a half-life of 4–10 min., removes from the blood up to half the injected antigen at the high rate of injection, or nearly all at the lower rate; the other has a half-life of 100–300 min.

By contrast, in normal rabbits there is a much slower disappearance of injected horse serum γ-globulins, a usual finding being that less than 30 % disappears in 1 hr. Graphical analysis again reveals two exponential processes with half-lives similar to those with immune rabbits, but in normal animals only about 15 % of the injected protein disappears in the rapid process. Some of the rabbits were killed 1 or 2 hr. after injection and the ^{131}I content of various tissues determined. The liver, lungs and spleens of the immune animals contained significantly more isotope than the corresponding organs of the normal animals. In the immune rabbits receiving the small injections as much as 90 % of the disappearing antigen was found in the liver.

Further experiments, correlating the amount of antigen precipitable *in vitro* by the serum anti-

bodies with that removed from the blood after injection, have shown that only about half the antigen disappearing *in vivo* can be precipitated *in vitro* by the antiserum. This suggests that there is some extra-vascular antibody which plays a considerable part in removing injected antigen from the blood.

Further Studies on the Composition of Sebum. Sebum-like Materials of Human Origin, and the Sebum of certain Animals. By V. R. Wheatley. (*Departments of Biochemistry and Dermatology, Medical College of St Bartholomew's Hospital, London*)

Earlier work (MacKenna, Wheatley & Wormall, 1950, 1952) has established the general composition of sebum (surface skin fat) obtained from the human forearm. Apart from true sebum there exist a number of materials that are alleged to originate from normal or modified sebaceous glands. Some of these materials (cerumen or ear wax, vernix caseosa, ovarian dermoid cyst lipid and epidermal cyst lipid) have been examined and their composition compared with that of sebum in order to decide whether they are in fact of sebaceous origin. Cerumen and vernix caseosa resemble sebum in composition; both, however, contain much more cholesterol than does sebum and while vernix caseosa contains less free fatty acids cerumen contains more than does sebum. Ovarian dermoid cyst lipid is different, it contains practically no free fatty acids and very much more 'eicosyl' esters than sebum. Squalene was found to be present in these three lipids but not in epidermal cyst lipid.

A study has also been made of the composition of the sebum of certain laboratory animals, namely, the rat, rabbit and guinea pig. The material examined was obtained by extracting the hair and skin of the intact animal with acetone immediately after death. Small amounts of free fatty acids were found in all specimens. The unsaponifiable matter from rat sebum was shown to contain aliphatic alcohols, cholesterol and other sterol-like substances, and 'isocholesterol'.

The author wishes to thank Dr Magnus Haines, of the Chelsea Hospital for Women, for supplying the ovarian dermoid cyst contents, and Dr M. H. Salaman, of the London Hospital, for a generous supply of rat sebum.

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A Substance from Animal Tissue which Stimulates Ketonuria. By H. B. Stewart and F. G. Young. (*Biochemical Laboratory, University of Cambridge*)

It was observed by Marks & Young (1939) that when dogs which had been rendered persistently diabetic by the administration of pituitary extract were fed a diet consisting almost entirely of beef suet ketonuria virtually disappeared. Addition of raw horse meat to the fat diet caused an immediate exacerbation of ketonuria while equivalent quantities of casein had no similar action. Addition of a concentrated extract of horse meat produced ketonuria under these conditions. Lazaris & Brzezhinskaya (1946) showed that when alloxan-diabetic rats were fed a 70% hog lard diet the addition of animal protein to the diet produced ketonuria while a similar quantity of vegetable protein did not.

The present experiments were undertaken to isolate the substance in the horse meat which induces ketonuria when fed with a high-fat diet. Normal or alloxan-diabetic male rats were given a high-fat diet containing protein either in the form of vitamin-free casein or washed heat-coagulated dried horse meat together with supplements of all the known vitamins except inositol. Under these conditions the body weight remains nearly steady for some months. Animals receiving casein exhibited no ketone bodies in the urine, while animals receiving horse meat showed mild to severe ketonuria. This response was demonstrable in both normal and alloxan-diabetic animals.

Administration of various types of tissue extracts to normal rats receiving the casein-containing high-fat diet caused the appearance of ketone bodies in the urine. With this test as a rough quantitative guide it has been possible to isolate a fraction from horse meat which is highly potent in the production of ketonuria under these conditions.

This material which possesses lipid-like solubilities is pure white and crystalline (m.p. 53°).

It is stable to hydrolysis by acids and alkali and is optically inactive. It contains C, H and O but shows no evidence of N, P, S or halogen. A spark spectrum reveals no metal component. The material behaves as a ketone and reacts with 2:4-dinitrophenylhydrazine, hydroxylamine and benzaldehyde. C 76·5 %, H 12·8 %, O 10·6 %. The infrared absorption spectrum, kindly carried out by Dr R. N. Haszeldine, of the Department of Chemistry, confirms the presence of a ketone group and the absence of other reactive groups. The structure and metabolic activity of this material are under investigation.

One of the authors (H. B. S.) would like to thank the Merck Postdoctoral Fellowship Board of the National Research Council of Canada whose support has made this work possible.

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DEMONSTRATIONS

A Windowless Geiger-Muller counter for ^{14}C and ^{35}S assay in solid Samples. By L. W. Blow and G. E. Francis

A bell-shaped Geiger counter of conventional design is mounted above a turntable containing three recesses for holding planchets, one of which is located under the counter. A continuous stream of helium-alcohol vapour mixture is kept passing through the counter in such a manner that a sample placed in the second recess is 'pre-flushed' with the gas mixture. On turning the turntable through 120°, a sample in the third recess is brought into the pre-flushing position, the pre-flushed sample is brought under the counter and may be counted immediately, and the counted sample, from the first recess, is removed.

Owing to improved geometry and absence of window absorption, the efficiency of the counter for ^{14}C or ^{35}S is approximately four times that of a conventional arrangement with a standard type mica window counter.

The Effect of Anionic Detergents and Related Compounds on some Enzymes. By E. D. Wills

An Improved Bioautographic Technique. By J. E. Ford and E. S. Holdsworth. (*National Institute for Research in Dairying, University of Reading*)

In our experience of the use of *Bacterium coli* and *Lactobacillus leichmannii* in bioautographic studies of vitamin B_{12}-like compounds, zones of exhibition are often ill-defined and difficult to detect, and at their best they are difficult to record photographically. The incorporation in the assay media of 2:3:5-triphenyl tetrazolium chloride offers a ready means of visualizing areas of bacterial growth, as they acquire a deep red pigmentation. The pigment is localized on the bacterial cells and does not diffuse into the surrounding agar.

For *Bact. coli*, we use the medium of Burkholder (1951), at single strength with 2 % agar added, and modified by the substitution of thiomalic acid for sodium thioglycollate. Flasks containing 150 ml. of medium are sterilized by autoclaving at 10 lb. for ten minutes and cooled to 47°. To each is added, before the inoculum, 4 ml. of a warmed 5 % solution of triphenyl tetrazolium chloride in sterile water.

We grow *Lb. leichmannii* in the medium of Skeggs, Nepple, Valentik, Huff & Wright (1950), modified by omitting the nucleotides, adjusting

the pH to 5·5 and adding 100 mg. ascorbic acid per 200 ml. single strength medium. With this medium it is necessary to avoid drastic heating, which gives rise to compounds capable of reducing the indicator. We therefore sterilize by filtration, warm to 47° and then add an equal volume of warm 4% agar before adding the 5% solution of indicator and a washed inoculum of the test organism.

We have used triphenyl tetrazolium chloride because it is commercially available and relatively inexpensive. A further useful property of this compound is that the bioautographic plate can be "developed" in a weak U.V. light, and very minute amounts of vitamin B_{12} thereby detected. Certain related compounds, however, offer advantages of increased stability and sensitivity; for example, 2:3:(4 styryl) 5-triphenyl tetrazolium chloride and the 'di-tetrazolium chloride'.

Apart from this application as a visual aid, we have demonstrated in preliminary experiments a quantitative relationship between the amount of vitamin B_{12} present on the filter paper and the amount of colour produced by bacterial growth. The coloured zones were cut from the agar and the dye eluted with tetrahydrofuran and measured colorimetrically. Colour density was proportional to the log concentration of the vitamin.

It seems probable that this improved technique may have more general applications as an aid to visualizing bacterial growth. For example, we have applied the method in plate assays, using *Lb. arabinosus* and *Saccharomyces carlsbergensis*.

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FORTHCOMING PAPERS

It is hoped to publish the following papers in the next issue of the *Biochemical Journal*:

The relation between riboflavin and tryptophan metabolism, studied in the rat. By FRANCINE CHARCONNET-HARDING, C. E. DALGLIESH and A. NEUBERGER

A method of measuring the yield of oxidative phosphorylation. By E. C. SLATER

Studies in carotenogenesis:

7. Further observations concerning the action of diphenylamine in inhibiting the synthesis of β-carotene in *Phycomyces blakesleeanus*. By T. W. GOODWIN, MALINI JAMIKORN and J. S. WILLMER

8. The carotenoids present in the basidiomycete *Dacromyces stillatus*. By T. W. GOODWIN

9. General cultural conditions controlling carotenoid (spirilloxanthin) synthesis in the photosynthetic bacterium *Rhodospirillum rubrum*. By T. W. GOODWIN and H. G. OSMAN

Respiratory granules of heart muscle. By K. W. CLELAND and E. C. SLATER

The effect of tonicity of the medium on the respiratory and phosphorylative activity of heart-muscle sarcosomes. By E. C. SLATER and K. W. CLELAND

Chemical action of X-rays on nucleic acids and related substances in aqueous systems. 1. Degradation of nucleic acids, purines, pyrimidines, nucleosides and nucleotides by X-rays and by free radicals produced chemically. By G. SCHOLES and J. WEISS

Urinary steroids:

1. Formation and determination of 3:5-dinitrobenzoates of alcoholic steroids. By A. E. KELLIE, E. R. SMITH and A. P. WADE

2. Chromatography of 3:5-dinitrobenzoates: application to the non-ketonic alcohol fraction. By A. E. KELLIE and A. P. WADE

The inheritance in the rat of the capacity to eliminate nicotinamide methochloride. By P. ELLINGER and P. ARMITAGE

Some properties of the bacitracin polypeptides. By G. G. F. NEWTON and E. P. ABRAHAM

Observations on the nature of bactracin A. By G. G. F. NEWTON and E. P. ABRAHAM

Anaerobic breakdown of phospholipins by brain suspensions. By G. H. SLOANE-STANLEY

The association of zinc and other metals with melanin and a melanin-protein complex. By J. M. BOWNESS and R. A. MORTON

Studies in vitamin A:

23. Vitamin A and its occurrence in *Amblystoma tigrinum*. By F. D. COLLINS, R. M. LOVE and R. A. MORTON

24. Spectroscopic examination of the lipids of two species of newts. By F. D. COLLINS, R. M. LOVE and R. A. MORTON

25. Visual pigments in tadpoles and adult frogs. By F. D. COLLINS, R. M. LOVE and R. A. MORTON

Metabolism of polycyclic compounds. 7. The metabolism of naphthalene, 1-naphthol and 1:2- dihydroxy-1:2-dihydro-naphthalene by animals. By E. BOYLAND and G. H. WILTSHIRE

Direct titrimetric determination of fluoride in natural waters. By AUDREY M. BOND and MARGARET M. MURRAY

3:5:3′-Triiodothyronine. 1. Isolation from thyroid gland and synthesis. By J. GROSS and ROSALIND PITT-RIVERS

Addendum—A crystallographic examination of triiodothyronine and some related compounds

3:5:3′-Triiodothyronine. 2. Physiological activity. By J. GROSS and ROSALIND PITT-RIVERS

Studies of the determination of the sequence of amino-acids in peptides and proteins. 1. The preparation, properties and chromatographic adsorption of the azobenzene-*p*-sulphonyl derivatives of various amino-acids. By H. M. FLOWERS and W. S. REITH

Purification and properties of a lysolecithinase from pancreas. By B. SHAPIRO

The acid polysaccharides of hog gastric mucosa. By H. SMITH and R. C. GALLOP

Addendum—Electrophoretic and ultracentrifugal examination of polysaccharides B and D. By B. R. RECORD and K. H. GRINSTEAD

The virulence-enhancing factor of mucins. 7. The remaining components of the 'third factor' involved in virulence enhancement. By H. SMITH, H. T. ZWARTOUW, R. C. GALLOP and PATRICIA W. HARRIS-SMITH

The separation of ketosteroids by partition chromatography using nitromethane as stationary phase. By J. K. N. JONES and S. R. STITCH

Role of acetoacetate in aggravating the disturbances in the carbohydrate metabolism of scorbutic animals. By M. C. NATH, V. K. SAHU and R. P. CHITALE

[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the authors.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **314th Meeting** *of the Biochemical Society was held in the Department of Physiology and Biochemistry, King's College, Strand, London, W.C.2, on Saturday, 17 January 1953, at 10.30 a.m., when the following papers were read:*

COMMUNICATIONS

The Higher Oxidation State of Metmyoglobin. By P. George and D. H. Irvine. (*Department of Colloid Science, University of Cambridge*)

We have shown by spectrophotometric titration that the intermediate compound formed from metmyoglobin (MetMb) and hydrogen peroxide is one oxidizing equivalent higher than MetMb and thus reacts like an iron compound with effective oxidation number +4 and not as a peroxide complex (George & Irvine 1951, 1952). It is not formed in a reversible equilibrium but in an irreversible reaction of the type

$$\text{MetMb} + H_2O_2 \rightarrow \text{Compound} + X, \qquad (1)$$

for example:

$$Fe_p^{3+} + H_2O_2 \rightarrow Fe_pO^{2+} + OH + H^+.$$

Further experiments have shown that

(1) Alkyl hydroperoxides behave similarly.

(2) Excess H_2O_2, as well as certain reducing agents added initially, reduce or eliminate the side reactions which occur when an approximately equimolar amount of H_2O_2 reacts with MetMb at pH's less than 8·0.

(3) The formation of the compound can be effected by oxidizing agents other than peroxides, e.g. potassium chloriridate, potassium molybdicyanide and sodium chlorite. Chloriridate acts at pH's alkaline to 6·8, but molybdicyanide only reacts above pH 11·0. Both these reactions are reversible. For instance, if the compound is formed with molybdicyanide at pH 11·0, and the pH changed to 10·0 by addition of a little acid or strong buffer, the compound is reduced back to MetMb.

(4) Whereas H_2O_2 and EtOOH only react appreciably with acid MetMb, with velocity constants of approximately 170 and 1700 M^{-1} sec.$^{-1}$ at 18° C., the reactions with chloriridate and molybdicyanide are extremely fast with velocity constants $> 10^5 M^{-1}$ sec.$^{-1}$ even with alkaline MetMb.

(5) The rate of reduction of the compound with ferrocyanide varies inversely with the hydrogen-ion concentration.

The compound itself is not responsible for the side reactions, which have been independently studied with methaemoglobin by Dalziel & O'Brien (1952), and their suppression by H_2O_2 or reducing agents is readily explained by competition for X in reaction (1). The formation of the compound by other oxidizing agents and its oxidizing capacity show conclusively that it is not a peroxide complex, and since chloriridate and molybdicyanide function by electron transfer it appears that the atoms necessary for its structure are already present in acid and alkaline MetMb and do not come from the oxidizing agent.

The hydrogen-ion dependency of the reduction of the compound suggests that H^+ is required to complete the stoicheiometry; for example:

$$Fe_pO^{2+} + H^+ + e^- \rightarrow Fe_p^{3+}OH^- \text{ (alkaline MetMb).}$$

A reaction of this type also accounts for chloriridate and molybdicyanide only forming the compound at pH's on the alkaline side of a certain threshold value. These pH values of 6·8 and 11·0 respectively are consistent with the compound having a pH-dependent redox potential with an E_2' of about 0·9 V., since chloriridate and molybdicyanide have pH independent redox potentials of 1·0 and 0·7 V.

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The Evolution of Dimethyl Sulphide by *Enteromorpha intestinalis*. Isolation of dimethyl-β-carboxyethyl sulphonium chloride from the alga. By R. BYWOOD and F. CHALLENGER. (*Department of Organic Chemistry, The University, Leeds*)

Following the isolation of dimethyl-β-carboxyethyl sulphonium chloride, $Me_2S^+(Cl^-)CH_2CH_2COOH$, from the red marine alga *Polysiphonia fastigiata* (Challenger & Simpson, 1948), further investigations have established the occurrence of this compound in the seaweed *Enteromorpha intestinalis*. On being gathered the weed spontaneously evolves dimethyl sulphide, probably by enzymic fission, as does *Polysiphonia fastigiata* (Haas, 1935). The sulphide was identified by m.p. and mixed m.p. of the mercurichloride ($2Me_2S.3HgCl_2$, m.p. 156°), sulphilimine ($Me.C_6H_4.SO_2N{\leftarrow}SMe_2$, m.p. 158°), platinochloride ($Me_2S.PtCl_2$, m.p. 160°), benzylsulphonium picrate ($Me_2.C_6H_5CH_2.S.C_6H_2(NO_2)_3O^-$, m.p. 134°) and trimethylsulphonium mercuriiodide ($(Me_3S)_2HgI_4$, m.p. 186°). The evolution of dimethyl sulphide from several plants on heating with sodium hydroxide has also been observed (Bywood, Challenger, Leaver & Whitaker, 1951). The precursor of this sulphide was extracted from the alga with ethanol, and after concentration of the extract was precipitated with saturated aqueous Reinecke's salt, $NH_4^+\left[Cr\begin{matrix}(SCN)_4\\(NH_3)_2\end{matrix}\right]^-$. The product was purified by repeated precipitation from acetone with ether.

The reineckate (decomp. 138°–140°) in aqueous acetone was shaken with excess of solid silver sulphate for 24 hr. A solution of the sulphonium sulphate was thus obtained which was freed from silver sulphate by evaporation to dryness under reduced pressure and extraction of the residue with warm methanol. This extract was evaporated, acidified with hydrochloric acid and precipitated with platinic chloride, giving a chloroplatinate (m.p. 197°, mixed m.p. with the chloroplatinate of the thetine from *Polysiphonia fastigiata*, 197°). This was shaken with freshly precipitated metallic silver and the minimum quantity of water, giving a solution of the chloride (Dudley, 1929) which after concentration gave a picrate (m.p. and mixed m.p. with the picrate of the thetine from *Polysiphonia fastigiata*, 132°) (Found: C, 36·60; H, 3·65; N, 11·90; S, 8·70; Calc. for $C_{11}H_{13}O_9N_3S$; C, 36·40; H, 3·60; N, 11·60; S, 8·80 %) and a styphnate (m.p. and mixed m.p. with the styphnate of the thetine from *Polysiphonia fastigiata*, 149°).

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Relative Utilization of Glucose and Acetate Carbon for Lipogenesis by Mammary Gland Slices, Studied with Tritium, ^{13}C and ^{14}C. By JUDITH H. BALMAIN, S. J. FOLLEY and R. F. GLASCOCK. (*National Institute for Research in Dairying, University of Reading*)

Mammary gland slices from lactating rats and ewes were incubated for 3 hr. in bicarbonate-saline (gas phase: 5 % CO_2, 95 % O_2) containing 1-^{13}C-2-tritioacetate (0·02 M) + ^{14}C-glucose (0·3 %). The total fatty acids were isolated as Ca salts and assayed for all three isotopes (Glascock, 1952).

Rat slices incorporated about eight times more glucose carbon and about six times less acetate carbon than did sheep slices. Sheep slices incorporated about the same small amount of glucose carbon both in the presence and absence of acetate; rat slices, however, incorporated more glucose carbon when acetate was absent than when it was present. This contrasts with our previous finding (Balmain, Folley & Glascock, 1952) that glucose stimulates the use of acetate carbon for lipogenesis in both tissues.

Insulin (1 i.u./ml.) increased to much the same extent the incorporation of acetate and glucose carbon by rat slices but had no effect on lipogenesis from either substrate in sheep slices, as found previously (Balmain *et al.* 1952) for the carboxyl carbon of acetate. Glycerol (0·2 %) similarly increased the incorporation of all three isotopes by rat slices, but its effect differed from that of insulin because it increased the utilization of acetate carbon significantly more than that of glucose. This

appears to rule out the possibility (see Balmain *et al.* 1952) that glycerol stimulates lipogenesis merely by removing fatty acids as glycerides from the site of synthesis.

These results further illustrate the striking metabolic difference between the mammary tissue of ruminants and non-ruminants, previously reported (Folley & French, 1950; Balmain *et al.* 1952). They suggest that the pro-lipogenic action of insulin is closely related to the utilization of carbohydrate, but it now seems doubtful whether this action is mediated through the formation of glycerol from glucose.

We are grateful to Dr D. H. Tomlin, Department of Physics, University of Reading, for assays of ^{13}C in the Agricultural Research Council's mass spectrometer and to Dr K. Hallas-Møller of Novo Terapeutisk Laboratorium, Copenhagen, for insulin free from hyperglycaemic factor. The ^{14}C-glucose was made from ^{14}C-starch kindly prepared for us by Dr Helen Porter, Institute of Plant Physiology, Imperial College, London. The skilled technical assistance of Mr B. W. E. Peaple is also acknowledged.

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The Dissociation Constants of some Conjugated Compounds. By D. Robinson, J. N. Smith and R. T. Williams. *Department of Biochemistry, St Mary's Hospital Medical School, London, W.* 2

Quick (1932) pointed out that many conjugated products formed during the so-called detoxication processes were highly acidic. However, the dissociation constants of very few of these compounds have been measured. We have recently measured the apparent dissociation constants of some thirty of these compounds. The pK_a of all these compounds were between 3 and 4. Twenty glucuronides had pK_a, 3·02–4·03; 3 mercapturic acids and 4 hippuric acids had pK_a *c.* 3·7, and DL-ornithuric acid had pK_a 3·7. At the pH of blood all these compounds would be completely ionized (99·9%) and therefore are readily excreted in the urine. It is already known that arylsulphuric acids are very strong acids.

In practically every case the conjugated products are much stronger acids than their precursors, e.g. phenol (pK_a, 10) and phenylglucuronide (3·42); benzene and phenylmercapturic acid (3·6); benzoic acid (4·2) and hippuric acid (3·7). An exception was found in the case of *o*-chlorobenzoic acid (pK_a 2·89) and *o*-chlorohippuric acid (3·79). The glycine conjugation of *o*-chlorobenzoic acid, however, is very small (5% in the rabbit) (Bray, Clowes, Thorpe, White & Wood, 1952) and in the case of this acid it appears unnecessary to produce a metabolite highly ionized at blood pH.

It is possible that if a compound has a pK_a of 3 or less, conjugation will not take place to any appreciable extent.

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The Urinary Porphyrins of Lead-poisoned Rabbits. By A. Comfort (*Department of Zoology, University College, London, W.C.* 1) and M. Weatherall (*Department of Pharmacology, The London Hospital Medical College, London, E.* 1)

We have investigated the ether-soluble porphyrins of lead-poisoned rabbit urine by lutidine-water chromatography on paper (Nicholas & Rimington, 1949, 1951), by esterification and chromatography on aluminium oxide columns (Nicholas, 1951) and by paper chromatography in two dimensions with kerosene-propanol and kerosene-chloroform mixtures (Chu, Green & Chu, 1951; Kench, Lane & Varley, 1952).

The esterified porphyrins are separable on alumina columns into five zones, corresponding in their elution-behaviour and in the position of the spots given by their saponification products on lutidine paper chromatography to at least five porphyrin

constituents. The lowest band consists of a trace of dicarboxylic porphyrin, the next contains mainly 3-carboxylic, the third mainly coproporphyrins, and the fourth and fifth a mixture of porphyrins in which a 6-COOH component appears to predominate. Lutidine chromatography of the free porphyrins derived from this zone occasionally shows a spot in the expected position of a 5-COOH porphyrin. We are inclined, therefore, to revise our previous statement that the porphyrin of lead-poisoned rabbit urine which moves more slowly upon lutidine paper chromatograms than coproporphyrin is predominantly a 5-COOH porphyrin (Weatherall & Comfort, 1952).

Chromatography of these ester fractions by the method of Chu *et al.* shows that the 3- and 6-COOH porphyrins are themselves inhomogeneous, and each exists in at least two forms. It is possible, from the analogy of their chromatographic behaviour to that of coproporphyrins I and III, that these represent the I and III series isomers of a tri- and a hexacarboxylporphyrin.

In addition to these substances, material from the top of the column, eluted with difficulty, was found to contain a porphyrin behaving on lutidine paper chromatography as a 4-COOH porphyrin, but on kerosene paper chromatography in a manner distinct from that of either coproporphyrin isomer, and resembling an 8-COOH porphyrin. The possibility that this unknown material is a column artifact is being investigated.

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Pasteur Effect and Aerobic Phosphorylation in Mammary Gland and Brain Homogenates.

By C. TERNER. (*National Institute for Research in Dairying, University of Reading*)

Homogenates in KCl of mammary glands of the lactating guinea-pig were incubated at 37° for 30–40 min. with reaction mixtures containing glucose, fructose-1:6-diphosphate, cozymase, nicotinamide, adenosine triphosphate, inorganic phosphate, $MgCl_2$, yeast hexokinase and either glycylglycine buffer pH 7·4 and cytochrome *c* (aerobic experiments), or bicarbonate buffer (anaerobic experiments).

Respiration (Q_{O_2}, 10–20 μl./mg. dry wt./hr.) was accompanied by vigorous phosphate uptake (Q_P, 10–20 μl. H_3PO_4/mg. dry wt./hr.), while only small amounts of lactic acid were formed ($Q^{O_2}_{\text{lactic acid}}$, about 2; $Q^{N_2}_{\text{lactic acid}}$, about 20 μl. lactic acid (determined chemically)/mg. dry wt./hr.). On addition of *p*-nitrophenol (2×10^{-4} to 4×10^{-4}M) the aerobic phosphate uptake was inhibited while lactic acid accumulation was increased to about 50% of the anaerobic level, with stimulation of respiration. In the presence of fluoride and added pyruvate the amounts of lactic acid formed under aerobic conditions were greater than in their absence, and the stimulating effect of *p*-nitrophenol on lactic acid accumulation was less clearly shown. Increasing amounts of fluoride (0·005–0·02M) caused increasing inhibition of respiration, accompanied by increased net phosphate uptake.

Iodoacetate (0·001M) inhibited respiration, phosphorylation and lactic acid formation. Arsenite (0·0025 M) increased aerobic lactic acid accumulation to approximately the same extent as *p*-nitrophenol, with inhibition of respiration and of aerobic phosphorylation. Similar results were obtained with brain homogenates under identical experimental conditions.

Although it is considered difficult to obtain broken-cell preparations which exhibit a natural Pasteur effect (see Dickens, 1951), a marked Pasteur effect has been observed in the experiments described. As in the yeast preparations used by Meyerhof & Fiala (1950), the Pasteur effect of mammary and brain homogenates is sensitive to inhibitors of oxidative phosphorylation. The results are in contrast to the findings of Reiner (1947) who reported high aerobic lactic acid formation and absence of a Pasteur reaction in brain homogenates. No effect of nitrophenols on the aerobic lactic acid formation in homogenates of brain and other tissues was observed by Clowes & Keltch (1951).

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The Reduction of Vinylacetate by *Clostridium kluyveri*. By J. L. Peel and H. A. Barker. (*Division of Plant Biochemistry, University of California, Berkeley*)

Previous work by Stadtman & Barker (1949*a*) has shown that preparations of *Clostridium kluyveri* catalyse the reduction of vinylacetate to butyrate by molecular hydrogen, although free vinylacetate itself is not an intermediate in the synthesis of butyrate from acetate and acetyl phosphate.

Certain atypical preparations of dried cells of this organism have now been obtained which are unable to carry out the reduction of vinylacetate unless an extract of boiled yeast is added. The yeast extract cannot be replaced by Co I, Co II or Co A, singly or together, or by the mineral constituents of the growth medium. Stadtman & Barker (1949*b*) previously observed that dialysed cell-free extracts of *Cl. kluyveri* required acetyl phosphate and yeast extract for the oxidation of butyrate. Further investigation of the system reducing vinylacetate has shown that the yeast extract can be replaced by catalytic amounts of acetyl phosphate or by acetyl Co A. The activating effect of acetyl phosphate continues as long as vinylacetate is present in the reaction mixture, but is quickly lost in the absence of vinylacetate.

On the basis of these observations, a mechanism is proposed for the reduction of vinylacetate which involves a cyclic series of transformations of the acyl derivatives of some cofactor, possibly Co A.

Attempts to demonstrate a Co A requirement for the reduction of vinylacetate by extracts of *Cl. kluyveri* were inconclusive. There were, however, indications of a requirement for some heat stable cofactor other than Co A, which may be identical with an unidentified factor shown by Korkes (1952) to be necessary for the hydrogenase system of *Cl. kluyveri*.

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Determination of Glucosamine in the Presence of Interfering Substances. By W. R. Smithies. (*Rothamsted Experimental Station, Harpenden, Herts*)

Zuckerkandl & Messiner-Klebermass (1931) estimated glucosamine by acetylation with acetic anhydride and sodium methylate to produce *N*-acetyl glucosamine, which, after treatment with alkali, gives a purple colour with Ehrlich reagent. This was superseded by the well-known method of Elson & Morgan (1933), which, however, is subject to interference, e.g. by amino-acid and sugar mixtures and by protein.

A method has been developed, similar to the original but more convenient and reproducible, as follows:

The solution, after evaporation to dryness in a vacuum desiccator, is treated with 2 ml. of methanol containing acetic anhydride (3 % v/v) and anhydrous sodium acetate (0·5 % w/v), then allowed to stand at least 4 hr. or preferably overnight. The solution is neutralized with 2 ml. 0·5N-Na_2CO_3, and acetyl glucosamine determinations are made on suitable aliquots after the method of Morgan & Elson (1934). Glucosamine hydrochloride carried through the above procedure is used as a standard, but may be replaced by a solution of *N*-acetyl glucosamine in aqueous methanol containing sodium acetate, acetic anhydride or acid and sodium carbonate in the corresponding proportions.

Aminoff, Morgan & Watkins (1952) have shown that when the Morgan & Elson (1934) method for the determination of *N*-acetyl amino-sugar is applied to *N*-acetyl chondrosamine, about five times as

much is required to give the same intensity as a given amount of *N*-acetyl glucosamine. This was found to be so when chondrosamine hydrochloride was acetylated under the above conditions.

Amino-acids and sugars do not interfere; for instance, the colour intensities of the standard and the blank were not affected by the addition of such a mixture which contained 450 μg. of apparent glucosamine hydrochloride when estimated by the Elson & Morgan (1933) procedure.

The method described has been found useful for the estimation of acetyl glucosamine in fungal mycelium autolysates. The acetylation here again prevents interference by amino-acids and sugars.

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The Volatile Fatty Acids in Fresh and in Fermented Kale. By A. J. G. Barnett, R. E. B. Duncan and (in part) D. K. Smith. (*Division of Agricultural Biochemistry, Department of Biological Chemistry, University of Aberdeen*)

During the course of a previous investigation into the production of volatile fatty acids during the fermentation of grass/water mixtures (Barnett & Duncan, 1952) it was observed that small amounts of free, or loosely combined, acetic acid—up to about 8 mg. % of the dry matter involved—were present in the fresh grass but no other lower fatty acids were noted while many experiments demonstrated the absence of lactic acid.

In a series of studies, using the technique of James & Martin (1952), on marrow-stem kale, it has been observed that in fresh kale small amounts of acetic acid and propionic acid are present. The quantities of these components involved are of the order of 74 and 8 mg. % respectively of the dry matter involved. In addition lactic acid (or acetaldehyde) was present to the extent of 54 mg. % of the initial dry matter.

During the fermentation of minced kale/water slurries under conditions of aeration, a slight drop in acetic acid concentration followed by a speedy production of this acid was observed. The propionic acid content rose slightly till the second day and then disappeared entirely. In an anaerobic mixture the results for propionic acid were the same, but the acetic acid level remained constant till its bacterial production started on the third day. If SO_2 was included in the initial mixture (6 g. SO_2 with 1400 g. of fresh kale and 2 l. of water) the lactic acid, acetic acid and propionic acid contents diminished to zero. These changes may be related to the cessation of plant cell respiration. By bacterial action, acetic acid is formed in large amounts in the aerated and anaerobic mixtures from the third day of fermentation onwards while in the slurry containing SO_2 only small amounts of acetic acid appeared.

Earlier investigation had shown, using the technique of Reid & Lederer (1952), that acetic, propionic, butyric and caproic acids could be isolated from the hydrolysed ether extract fraction of grass. The corresponding ether extract from dried fresh kale, after shaking with $NaHCO_3$ and removal of the ether, gave on hydrolysis with alcoholic KOH and subsequent analysis, the following acids: formic acid 2·75 mg. %, acetic acid 8·07 mg. %, propionic acid 0·66 mg. %, *n*-butyric acid 0·78 mg. %, *n*-caproic acid 0·65 mg. % of initial dry matter.

Whether these acids are actually present in ester form in the plant or whether they are in fact formed by decomposition of a hypothetical organic lactate under conditions of alkaline hydrolysis, is not yet known.

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The Vitamin E Activity of Methylene Blue. By T. Moore, I. M. Sharman and R. J. Ward. (*Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*)

Dam has reported that various natural and artificial redox substances, including methylene blue, protect rats and chicks against most of the abnormalities caused by deficiency of vitamin E (Dam, Kruse, Prange & Sondergaard, 1951; Christensen & Dam, 1951; Aaes-Jorgensen, Dam & Granados, 1951; Dam & Granados, 1951, 1952; Dam, Prange & Sondergaard, 1951, 1952*a*, *b*). In order to obtain independent evidence on this finding the potency of methylene blue was studied in rats given from weaning a basal diet of casein (vitamin-free) 25%, sucrose 50%, lard 10%, dried brewer's yeast 10% and salt mixture 5%. Doses of 1000 i.u. of vitamin A were administered weekly, and vitamin K was also provided.

It was found that some of the lesions associated with vitamin E deficiency were prevented, but that others were unaffected by methylene blue. Thus when 0·12% of the dye was included in the diet from the commencement of the trials there was complete protection against brown discoloration of the uterus and also against cloudy swelling of the convoluted tubules of the female kidney. In each instance this protection was maintained for a period of several months. Methylene blue was also effective in increasing the storage of vitamin A in the liver.

In the prevention of resorption gestations in rats depleted of vitamin E, however, methylene blue was ineffective, whether given to the rats from weaning or from mating. Even after resorption gestations the rats which had been dosed with methylene blue had normal kidneys, and in those which had been given the dye from weaning the uteri were normal in colour. Atrophy of the testes was not prevented by methylene blue. As previously reported (Heard, Moore & Sharman, 1952), the red blood cells of rats given methylene blue were not protected from haemolysis by dialuric acid. In albino rats depigmentation of the incisor teeth was not prevented by the dye, but there was a delay of 1–2 weeks in the disappearance of the brown colour.

The ability of methylene blue to protect against some lesions, but not against others, suggests that the effects of vitamin E deficiency may be grouped together according to whether they are due primarily to the absence of the vitamin, or alternatively to the secondary action of abnormal metabolic products.

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The Enzymatic Decarboxylation of γ-methylene glutamic acid. By L. Fowden (*Botany Department, University College, London*) and J. Done (*Human Nutrition Research Unit, Medical Research Council Laboratories, Holly Hill, Hampstead, N.W. 3*)

The isolation of γ-methylene glutamine (I) and γ-methylene glutamic acid (II) from groundnut plants has been described (Done & Fowden, 1952). A third unsaturated amino-acid (compound IV in the paper cited) was also reported and now appears to be the α-decarboxylation product of II. Compounds, I, II and IV also appear to be present in extracts of tulip leaves (see also Steward & Thompson, 1950).

IV occurs only in small amounts in the plant extracts examined and has not been isolated in pure form. On chromatograms it appears as a yellowish brown spot moving slightly faster than valine in *n*-butanol-acetic acid (Partridge, 1948), and can easily be separated from the more slowly moving spot of allylglycine, which would arise by elimination of CO_2 from the ω-COOH group of II. Plant extracts capable of decarboxylating II have been prepared and the product is inseparable from IV on chromatograms.

Significant decarboxylation was first observed using extracts of the fruits of a *Capsicum* species

buffered at pH 5·6. The CO_2 produced in 4 hr. was 63·0 % of the calculated amount for complete α-decarboxylation, whilst IV was produced in amounts equivalent to 60·7 % of II used. Under the same conditions *l*-glutamic acid was completely decarboxylated at an initial reaction rate 3·4 times greater than that observed for II. When an extract of barley roots prepared by the $(NH_4)_2SO_4$ precipitation method of Beevers (1951) was used the corresponding figures after 165 min. at pH 5·6 were: 65·3 % decarboxylation as measured by CO_2 evolved and 60·5 % as determined by IV produced.

In further experiments with barley-root extracts, the initial rates of decarboxylation of II and *l*-glutamic acid were compared at different pH's. The optimum pH for both substrates appeared to be identical, whilst the rates of decarboxylation of *l*-glutamic acid were always 2·1–2·6 times the corresponding rates observed for II over the pH range 4·0–7·0.

The results seem to indicate that a glutamic decarboxylase may be responsible for the decarboxylation of II. This idea is strengthened by the fact that II is completely decarboxylated by *Clostridium welchii* S.R.12 (kindly supplied by Dr E. F. Gale).

Our thanks are due to Mr V. Moses for his assistance in the manometric experiments and to Miss A. E. Wright for technical assistance.

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Separation of Uroporphyrin Esters I and III by Paper Chromatography. By J. E. Falk and A. Benson. (*Department of Chemical Pathology, University College Hospital Medical School, London, W.C.* 1)

Chu, Green & Chu (1951) have described a paper chromatographic method for separating the methyl esters of protoporphyrin, coproporphyrins I and III and uroporphyrin I, but not uroporphyrin III. We now describe a paper chromatographic method which achieves this separation. By a combination of the methods, all five main natural porphyrins may be separated on a single paper square.

Chromatography was by the ascending method, at 22–23°, on 22 cm. squares of Whatman no. 1 paper. The porphyrin esters were applied from freshly prepared acetone solutions. The reference samples were: uroporphyrin I ester from a case of congenital porphyria, m.p. 293° (Rimington & Miles, 1951), and uroporphyrin III ester from Turacin, m.p. 264° (Nicholas & Rimington, 1951). Kerosene was heating-paraffin, distilled over $CaCl_2$, b.p. 190–250°. Chloroform B.P. was washed with water and dried; for procedure (1), 1 % (v/v) of ethanol was added. Dioxan was purified by the method of Eigenberger (1931).

Separation of uroporphyrins I and III. (1) The paper was developed, in an atmosphere of chloroform, by kerosene-chloroform 4:6 (v/v), until the porphyrin spots had moved half-way up the paper, which was then dried. The base of the paper was cut off ½ in. below the spots. The two isomers move at the same rate in this development, which merely moves them away from the base-line and from non-mobile impurities.

(2) The cut-down square was developed in the same direction as before, in an atmosphere of dioxan, with kerosene-dioxan 4:1·5 (v/v), the solvent being allowed to reach the top of the paper. The R_f values, calculated from the position of the spots after procedure (1), are approximately 0·03 and 0·4 for uroporphyrin esters I and III respectively. In mixtures, 15 % of either isomer was easily distinguished.

Separation of the five main natural porphyrins. After development by the method of Chu *et al.*, uroporphyrins I and III remained together near the base-line. The paper was now cut off parallel with the base-line, just above the uroporphyrins, leaving the separated coproporphyrins I and III and protoporphyrin on the upper portion. The lower portion was then developed as in (2) above, but in a direction at right angles to the 'Chu' developments. Uroporphyrin I ester barely moves, uroporphyrin III ester separating from it with an R_f of approximately 0·45.

The method has proved useful for routine examination of porphyrin mixtures from natural sources.

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The Interaction of Silicic Acid with Monolayers of a Protein. By P. F. HOLT and J. E. L. BOWCOTT. (*University of Reading*)

Silicon is normally present in animal tissues (King, Stantial & Dolan, 1933). A part of the tissue silicon is combined in structures which are soluble in ethanol and which have been studied by Holt & Yates (1953). The silicon which is present in complexes insoluble in ethanol appears to be associated with protein. A study has, therefore, been made of the interaction between silicic acid sols and monolayers of protein at air-water interfaces. Charged films of amines adsorb silicic acid from dilute solutions, and become rigid when the acid polymerizes (Wolstenholme & Schulman, 1950). In this present work we have used more concentrated silicic acid solutions (0·01–0·1 M) to investigate the state of a protein monolayer when it is adsorbed by or adsorbs silicic acid micelles.

When spread on phosphate buffer sub-solutions, bovine serum albumin monolayers are condensed, at zero compression, to areas of about 1 m.²/mg. On sub-solutions containing silicic acid, monolayers compressed exactly one minute after spreading condensed to areas less than this value. The actual extent of contraction depended upon the concentration, pH and age of the silicic acid solutions.

Between pH 4 and 7, silicic acid aggregates to micelles at a rate dependent upon the concentration. The older solutions which contain larger micelles cause greater contractions. When completely contracted, that is, condensing to about 0·4 m.²/mg. at zero surface pressure, the films appear to be in a state similar to a normal protein that has been compressed by more than 20 dynes/cm. They are rigid and incompressible. However, in this case the films are not solid unless subjected to prolonged compression. At low pressures they again return to large areas indicating that the protein molecules remain independent and that adsorption is rapid and occurs immediately after spreading, before the films are compressed.

Work is continuing with more simple compounds, to investigate the structure of the adsorption complex.

This work was supported by a grant to one of us (J.E.L.B.) given by the Department of Scientific and Industrial Research.

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The Qualitative Determination of Higher Fatty Acids by Means of String Electrophoresis. By A. J. G. BARNETT, H. LEES and D. K. SMITH. (*Department of Biological Chemistry, University of Aberdeen*)

Methods exist for the separation of C_1–C_{12} fatty acids. The method of Howard & Martin (1950) can be used for the estimation of acids higher than C_{12}, but elaborate apparatus and series of solvents are required. The following procedure is suggested as a means of identification of these higher fatty acids, and it is conceivable that the method can be adapted to quantitative work and other types of separation.

Candle-wick thread, 86 cm. in length, is whipped at each end with cotton and extracted for 8 hr. by boiling with distilled water which is changed periodically. After drying, the thread is extracted for 4 hr. with ether and placed in a solution (0·25% w/v) of paraffin wax (m.p. about 60°) in petroleum ether (40–60°). The thread, which after solvent evaporation contains about 0·019 g. of paraffin wax, is passed through a glass tube (bore, 4 mm. × length 54 cm.) with a right-angled bend 10 cm. from one end. The thread protrudes about 5 cm. from the longer arm and the tube is clamped vertically, the shorter arm being horizontal. The thread from the short arm is immersed in pure 3N-NH_4OH. Platinum wire is wound round the string at each end, the upper being the (+)ve electrode. The electrodes are fixed so that the (+)ve one is against the short arm while the (−)ve one is 2 cm. from where the thread leaves the tube. Siphoning of the NH_4OH proceeds overnight and loading is then accomplished by pulling back the thread 1 cm. at the upper end and placing on it 0·05–0·10 ml. of the solution of fatty acid in 3N-NH_4OH.

A current of 2 mA. at 300 V. d.c. was passed through a battery of five strings set up in parallel and loaded, as described, with stearic, palmitic,

myristic and lauric acids (100–200 μg. each) and a mixture of all four. The eluates were collected in tubes (10 × 100 mm.) charged every hour for 36 hr. The tubes were then heated to dryness at 80° and, after cooling, 1 ml. of formalized brom-cresol purple indicator (pH 7·3) was added to each and the colour change noted. Stearic palmitic, myristic and lauric acids appear approximately nine, thirteen, twenty-two and thirty-one hours respectively from a mixture.

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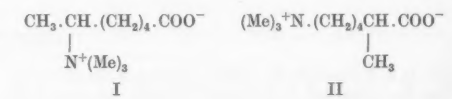
A new Chemical Degradation of Cholesterol. By J. W. Cornforth, G. D. Hunter and G. Popják. (*The National Institute for Medical Research, Mill Hill, London*)

For a study of cholesterol biosynthesis from isotopically labelled precursors a new method for the partial degradation of the ring structure of cholesterol was developed. Eight carbon atoms, carbons 1, 2, 3, 4, 5, 6, 10 and 18 can ultimately be obtained individually as CO_2.

In one of our methods cholesterol was converted to cholest-5-ene and the latter ozonized. Reduction of the ozonide with zinc powder and acetic acid gave 5:6-*seco*-cholestan-6-al-5-one, which on treatment with sodium ethoxide recyclized to B-*nor*cholestan-6-al-5:7-ene. Ozonization, followed by reduction with zinc and acetic acid, of the nor-aldehyde gave 5:6-*seco*-cholesta-5:7-dione-6-carboxylic acid, which was readily decarboxylated by heating to 200°, or by boiling with aniline, to give carbon atom 6 of cholesterol as CO_2. The decarboxylated product, 5:6-*seco*-B-*nor*cholestan-6-al-5-one, gave on pyrolysis at 450–500° with K_2CO_3 2-methyl-*cyclo*hexanone.

2-Methyl*cyclo*hexanone on treatment with HN_3 gave a mixture of two isomeric lactams, which were hydrolysed to ϵ-amino-heptanoic and α-methyl-ϵ-aminohexanoic acids. Methylation of the two amino-acids with methyliodide in methanol gave the corresponding betaines (I) and (II), which on heating with KOH to 150–200° readily lost trimethylamine. Rearrangement of the initially formed double bond takes place on raising the temperature to 300°; when the double bond reaches a position $\alpha\beta$ to the carboxyl group, an oxidative fission occurs. Thus I splits into acetic (carbons 4 and 5 of cholesterol) and valeric acids (carbons 18, 10, 1, 2 and 3 of cholesterol); and II gives propionic (carbons 18, 10 and 5) and butyric acids (carbons 4, 3, 2 and 1 of cholesterol). The four acids can be separated from the reaction mixture by partition chromatography on suitable columns and subjected further to carbon-by-carbon degradation by standard procedures.

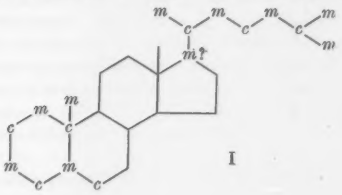


I II

Distribution of Acetate Carbon in the Ring-structure of Cholesterol. By J. W. Cornforth, G. D. Hunter and G. Popják. (*The National Institute for Medical Research, Mill Hill, London*)

The method described in the previous communication has been applied to two samples of ^{14}C-cholesterol synthesized by rat-liver slices *in vitro* from [*carboxy*-^{14}C]-acetate and [α-^{14}C]-acetate respectively. 7 μc. of [^{14}C]-cholesterol synthesized from [*carboxy*-^{14}C]-acetate and 13 μc. synthesized from [α-^{14}C]-acetate were available for degradation. Eight carbon atoms, numbers 1, 2, 3, 4, 5, 6, 10 and 18, were obtained as $BaCO_3$ and their radioactivities measured. When [*carboxy*-^{14}C]-acetate was the precursor of the labelled cholesterol, carbon atoms 2, 4, 6 and 10 contained ^{14}C in equal amounts, while carbons 1, 3, 5 and 18 were inactive. On the other hand, in [^{14}C]-cholesterol biosynthesized from [α-^{14}C]-acetate high degree of labelling was found in carbon atoms 1, 3, 5 and 18 and a smaller one in carbons 2, 4, 6 and 10, the specific activity of these latter carbons being about 20 % of the first named group. The latter result can be explained by the production, through the tricarboxylic acid cycle, of [*carboxy*-^{14}C]-acetate from [α-^{14}C]-acetate before incorporation into cholesterol.

It is concluded that carbon atoms 2, 4, 6 and 10 of cholesterol are derived from the carboxyl carbon, and carbon atoms 1, 3, 5 and 18 from the methyl carbon of acetate. It should be mentioned also that the specific activity of the total carbon of cholest-5-ene agreed with the assumption that 12 carbons of cholesterol are derived from the carboxyl-carbon and 15 from the methyl carbon of acetate, thus confirming the results of Little & Bloch (1950). Wüersch, Huang & Bloch (1952) have recently identified the origin of the carbons in the iso-octyl side-chain of cholesterol from acetate. Their results and ours put together give the distribution pattern of acetate carbon in cholesterol as shown in the formula (I) in which *c* denotes a carbon derived from the carboxyl and *m* a carbon derived from the methyl carbon of acetate.



I

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On the Determination of the Sequence of Amino-acids in Peptides. By W. S. Reith and N. M. Waldron. (*Organic Chemistry Department, The University, Leeds*)

3 : 5 - Dinitro - 4 - dimethylamino - phenyl - *iso* - thiocyanate (DDPT) was synthesized. Owing to the configuration of the substituents in the benzene ring the substance is deeply coloured; it reacts at room temperature with *N*-terminal amino-acids of peptides dissolved in water-acetone (1 : 3, v/v) at pH 8–9. For instance, alanyl-glycyl-glycine yielded 3 : 5-dinitro - 4 - dimethylamino phenyl - thiocarbamyl-alanyl-glycyl-glycine (DDPT-alanyl-glycyl-glycine) as orange needles m.p. 132–5° (d.) in 96 % yield.

The solution of this substance in water-acetic acid (80 : 20, v/v) when kept for 24 hr. at 40° C, yielded glycyl-glycine (85 % of theor.), and 3-(3 : 5-dinitro-4-dimethylamino-phenyl)-5-methyl-2-thio-hydantoin (DDPT-alanine-hydantoin) as orange crystals m.p. 222–3° (d.) in 82·5 % yield.

The solution of the residual peptide, glycyl-glycine, when treated with a solution of DDPT in the same way as described above, yielded DDPT-glycyl-glycine; this substance when dissolved in the water-acetic acid solution, and kept for 5 days at 40° yielded glycine and DDPT-glycine-hydantoin as orange crystals m.p. 252–3° (d.) in 84 % of the theoretical yield. The residual glycine was treated in the same way as indicated for glycyl-glycine, when DDPT-glycine-hydantoin was obtained.

Mixtures of DDPT-hydantoins of various amino-acids can be separated by adsorption chromatography on columns of alumina pre-treated with methanol-acetic acid (90 : 10, v/v). As these substances are deeply coloured, the development of the chromatograms can easily be controlled, and very small amounts can be estimated spectrophotometrically.

The DDPT-hydantoins have characteristic melting points. However, an independent assessment of their identity can be obtained by hydrolysis to the parent amino-acids, and the subsequent use of paper chromatography. We found that the hydrolysis can be most conveniently effected by heating those hydantoins with a mixture of formic, acetic and perchloric acids as used by Hanes, Hird & Isherwood (1952) for the hydrolysis of peptides.

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The Distribution of Arylsulphatase in the Rat-liver Cell. By K. S. Dodgson, B. Spencer and J. Thomas. (*Physiology Institute, Newport Road, Cardiff*)

Differential centrifugation of sucrose homogenates of rat liver has been used to fractionate the various cytological components of this tissue. Using isotonic sucrose (Schneider, 1948), the arylsulphatase activity (Dodgson, Spencer & Thomas, 1952) of the different fractions, expressed as percentages of the whole homogenate, was: nuclear 12·7, mitochondria 9·5, microsomes 58·6 and supernatant 3·1. With hypertonic sucrose (Hogeboom, Schneider & Pallade, 1948) the figures were: nuclear 17·8, mitochondria 25·0 and microsomes plus supernatant 48·3. Sucrose had no effect on the enzyme.

The activity of the nuclear fractions can be attributed to undegraded liver cells and contaminating granular material; the latter being more prominent in the nuclear fraction from hypertonic than from isotonic sucrose. Blood cells and nuclei contributed little to the activity of this fraction. Isolated nuclei had negligible activity. It is obvious therefore that the arylsulphatase of rat liver is localized mainly in the granular material of the cytoplasm, the major portion being associated with the so-called microsomes. The partition of the enzyme between mitochondria and microsomes varies with the fractionation medium. This variation may be due to redistribution of the arylsulphatase amongst the cytoplasmic particles during the fractionation procedures. Alternatively, there may be some breakdown of mitochondria in isotonic sucrose since this medium does not preserve the morphological integrity of these granules (Schneider, 1948). Dr A. B. Roy (private communication) has previously found mouse liver mitochondria to possess arylsulphatase activity. At pH 6·8 in isotonic sucrose there is virtually no arylsulphatase in solution.

According to Bernstein & McGilvery (1952) the enzyme of rat liver responsible for the synthesis of arylsulphates is almost entirely associated with the supernatant of an isotonic KCl homogenate after the removal of nuclear matter at 2000 *g* and granular material at 18,000 *g*. Both the low- and high-speed residues had negligible synthesizing activity. We have repeated this fractionation and have tested each fraction for arylsulphatase activity. It has been found that the hydrolysing enzyme was activated by KCl and was distributed between the high and low speed residues whilst the supernatant had only approximately 6% of the total activity. These results suggest that the enzymes which synthesize and hydrolyse arylsulphates in the mammalian body are different.

We wish to thank the Cardiff Pure Ice and Cold Storage Co., for generously placing a cold room at our disposal.

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The Effect of Nucleic Acids on Tryptophanase Formation. By A. A. Hakim and F. C. Happold. (*Department of Biochemistry, School of Medicine, Leeds*)

Deoxyribonucleic acid (DNA) prepared from cells of *Escherichia coli* increases the rate of tryptophanase formation during adaptation. Within limits the increase is roughly proportional to the amount of DNA added to the system. Similar results were obtained with industrial DNA. Thymine and ribonucleic acid (RNA) are without effect upon adaptation, but the addition of adenine to suspensions of *Esch. coli* to a final concentration of 0·006 % (w/v) delays adaptation for periods of 4–5 hr.

The Preliminary Isolation and Characterization of Human Serum Cholinesterase. By R. G. O. Kekwick, M. E. Mackay and N. H. Martin. (*St George's Hospital and the Lister Institute*)

Ether fractionation of human serum by techniques developed at the Lister Institute (Kekwick, Mackay & Record, 1946; Kekwick & Mackay, 1948) divides the serum proteins into a fraction containing the γ-globulin together with α- and β-lipoproteins, and a crude albumin fraction containing albumin contaminated by lipoid-poor α- and β-globulins. The first fraction, and pure albumin separated from the crude albumin fraction, have minimal esterase activity. A further study of the globulins separated

from the crude albumin fraction suggested that the main cholinesterase activity was associated with the α-globulin. Concentration of the cholinesterase activity by ethanol fractionation in an α-globulin rich fraction was reported by Surgenor (1949).

A fraction containing 15% α-globulin was obtained from the crude albumin. Further precipitation gave a preparation containing 40% α-globulin. This has been purified again to give a preparation containing 60 % α-globulin with an activity 48 times greater than human serum (7400 CO_2 μl/hr./mg. N). A close parallel was observed between the α-globulin nitrogen and the actylcholine splitting of these fractions.

Examination of these purified materials has been commenced and their behaviour has been related to that of normal serum. Measurement of esterase activity in the Warburg apparatus at different temperatures showed that for whole serum, as for the 15 and 40 % α-globulin fractions, the optimal temperature for hydrolysis of acetyl choline lay between 47 and 50°. The critical increment obtained by plotting $\log_{10}$ rate of hydrolysis against the reciprocal of absolute temperature, was 6500 kcal./g.mol. in each case. This suggests that in respect of this property, the active protein has undergone no modification during fractionation. It is interesting to compare these figures with those of horse serum reported originally by Glick (1939) and confirmed by ourselves. Horse serum has optimal temperature of about 40° C. for the hydrolysis of acetyl choline, and a critical increment of 5000 kcal./g.mol.

Comparisons of the hydrolysis rates of triacetin by these purified fractions showed a 13-fold increase in triacetin activity of the final fraction over whole serum compared with a 48-fold increase in acetylcholine activity. The latter results suggest that two sites are concerned with the esterase activity or more probably two proteins.

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The Isolation of a Compound of Uridine Diphosphate and Glucuronic Acid from Liver. By G. J. Dutton* and I. D. E. Storey.† (*Departments of Biochemistry and of Surgery, University of Edinburgh*)

We have previously reported (Dutton & Storey, 1951) that liver contains a thermostable factor necessary for the formation of *o*-aminophenylglucuronide from *o*-aminophenol in liver homogenates, and that the factor cannot be replaced by a wide variety of co-factors or metabolic intermediates. Further studies have shown that β-D-glucuronic acid-1-phosphate is without effect (cf. Touster & Reynolds, 1952), and that the reaction differs greatly from the formation of *o*-aminophenylglucuronide by liver slices (Storey, 1950) in that it is anaerobic, and uninfluenced by bicarbonate or sulphate ions.

The factor has been obtained in a highly purified form from a trichloroacetic acid extract of rabbit liver by precipitation of the ethanol-insoluble barium salts, ion-exchange chromatography and re-precipitation with barium. At this stage paper chromatography showed that the activity was always associated with phosphate and with ultraviolet absorption, the latter being due almost entirely to a uracil derivative. Mild acid hydrolysis liberated a reducing agent, its rate of release running parallel to the loss of activity of the substance. A positive Tollens naphthoresorcinol reaction was also obtained, the material responsible being approximately equivalent in amount to that of the reducing agent, if calculated as glucuronic acid.

Further purification could be effected by paper chromatography in isopropanol—1 % ammonium sulphate solution (Anand, Clark, Hall & Todd, 1952) when two components were observed. Both contained uracil and phosphorus, but only the slower-moving component was active and contained the naphthoresorcinol-positive substance. In the active compound half the total phosphorus was split off in 12 min. in N-HCl at 100°, the remainder proving much more resistant. Uridylic acid, total P, 'labile' P and the naphthoresorcinol-positive substance (calculated as glucuronic acid) were present in the ratios 1:2:1:1.

* In receipt of a grant from the Medical Research Council.

† External Staff, Medical Research Council.

When incubated with liver homogenates and *o*-aminophenol or (−) menthol, approximately one equivalent of the corresponding glucuronide is formed for each equivalent of uridylic acid in the purest preparation, the glucuronides being characterized by enzymic and chromatographic methods.

Recent work (Caputto, Leloir, Cardini & Paladini, 1950; Park, 1952) has demonstrated the natural occurrence of various derivatives of uridine diphosphate. The marked resemblance of these compounds to the newly isolated factor suggests that it may have the structure 'uridine-diphosphate-glucuronic acid'. Further evidence will be presented.

We wish to thank Dr O. Touster for the gift of the barium salt of β-D-glucuronic acid-1-phosphate.

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A Serologically Active Polysaccharide from the Capsules of Amylolytic Rumen Streptococci.

By P. N. Hobson and Margaret J. MacPherson. (*The Rowett Research Institute, Bucksburn, Aberdeenshire*)

In the course of some studies on amylolytic streptococci isolated from the rumen of the sheep (MacPherson, 1952) attempts were made to assign the bacteria to a particular group by the Fuller and Lancefield methods (Fuller, 1938; Lancefield, 1925). The antigenic extracts obtained by these methods contained both protein and carbohydrate and gave somewhat different results in the serological tests. Other methods of preparing serologically active specific polysaccharides were then investigated. A polysaccharide extracted from the metabolic liquid after growth of the cells in glucose-Lab-Lemco peptone medium had a higher serological activity and a lower protein content. The most active preparation was, however, obtained by removing the capsules from the washed cells by heating at 100° with acetate buffer, pH 2, containing a little Tween 80 (Honeywell and Stein, Ltd., London), the dissolution of the capsules being followed by frequent microscopic observations. After removal of protein by the method of Sevag (1934) a polysaccharide was obtained from the solution which had the composition; N, 0·68%; ash, 7·2%, reducing sugars (as glucose) after hydrolysis for 3 hr. in 1·5N-H_2SO_4 at 100°, 50·3%, after 6 hr. 58·2%; rhamnose, 17·6%; uronic acid, 22%. Hexosamine could not be detected, and galactose and rhamnose, with a trace of glucose, were found on chromatograms of the hydrolysate, accounting for the low reducing sugar value. The polysaccharide, which was entirely soluble in cold water, gave a precipitin reaction at a concentration of 18×10^{-5} mg./ml. with an antiserum prepared by injecting formolized suspensions of the whole cells into rabbits.

This capsular polysaccharide would appear to differ from any bacterial polysaccharide so far described, although it resembles in some respects those obtained from some types of pneumococci (Stacey, 1952).

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The Relation of Glutamine to the Synthesis of Hyaluronate or Hyaluronate-like Substances by Streptococci. By D. A. Lowther and H. J. Rogers. (*National Institute for Medical Research, Mill Hill, London, N.W. 7*)

It has been known for some time (Kendall, Heidelberger & Dawson, 1937; Seastone, 1939; McClean, 1941) that certain strains of *Streptococcus haemolyticus* can synthesize hyaluronate when growing in laboratory media. The polysaccharide thus formed appears to be similar to, if not identical with, that isolated from mammalian sources. In such cultures the hyaluronate may occur both as capsular material around the cells and free in the culture fluid (Seastone, 1939; McClean, 1941). It was decided that this bacterial system might be the most satisfactory for studying the mechanism of synthesis of the so-called mucopolysaccharides.

It was found that well washed organisms of a mucoid strain of *Strep. haemolyticus* of Lancefield Group A (kindly supplied by Dr R. E. O. Williams) harvested from a culture grown in buffered broth serum glucose medium, would synthesize hyaluronate when suspended in a solution containing: 0·028M-glucose, 0·11M-Na β-glycerophosphate as buffer, 0·025M-inorganic phosphate (as a mixture of sodium and potassium hydrogen phosphates), 0·011M-thioglycollic acid and diluted bacteriological broth. The amount of hyaluronate which appeared in the supernatant fluid after 2 hr. incubation of such a suspension at 35° under an atmosphere of air, estimated by turbidity test using the conditions of Tolksdorf, McCready, McCullagh & Schwenk (1949) and by glucosamine determinations made by the method of Morgan & Elson (1933) using acid hydrolysates, corresponded to about 0·6 mg./mg. bacterial N. Little or no hyaluronate was formed in the absence of broth. Hyaluronate did not appear to remain attached to the organisms when these were treated by the method used by Seastone (1939). The broth in the suspending fluid could be replaced by glutamine at a final concentration of $5{\cdot}37 \times 10^{-3}$M and under some conditions by asparagine. Of 24 amino-acids and other amides, only serine and argine showed slight activity.

When cells were examined within 24 hr. of harvesting and washing it was found that glutamine could be replaced by HN^+_4 (final concentration $1{\cdot}75 \times 1{\cdot}0^{-2}$M) added as NH_4Cl. If, however, the cells stood in sodium β-glycerophosphate solution (5 g./100 ml.) for about 4 days at 0–4° and were then centrifuged and resuspended in fresh buffer, they were considerably less active in the presence of NH^+_4 than with glutamine. When the 'aged' cells were incubated with glutamate *and* ammonia the amount of hyaluronate was much increased, being almost as great as that in the presence of glutamine; glutamic acid alone did not allow significant synthesis. Synthesis also took place with ammonia using 'aged' cells if the original supernatant was used as suspending fluid. The present evidence suggests that glutamine may play an essential part in the synthesis of hyaluronate, possibly by transference of its amide nitrogen.

The evidence that the substance in the supernatant giving turbidity with acidified serum is hyaluronate is as follows: (1) it is destroyed by streptococcal hyaluronidase prepared as previously described (Rogers, 1946); (2) glucosamine estimations on acid hydrolysed supernatants parallel the amount expected on the basis of the turbidity tests; and (3) small amounts of material isolated from supernatants have a glucosamine/N ratio of 10·55 and some of the general properties of hyaluronate.

This work has so far been confined to the study of one strain of *Strep. haemolyticus*.

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The Action of Plant Extracts on β-Glucuronidase. By C. A. Marsh and G. A. Levvy. (*Rowett Research Institute, Bucksburn, Aberdeenshire*)

Hydrolysis of phenolphthalein glucuronide by animal β-glucuronidase was strongly inhibited by aqueous extracts of the foliage of all plants examined, and also by extracts of cereal husks and liquorice root. In all instances in which it was further studied, the active principle was dialysable, fairly stable to acid and alkali, and moderately thermostable. It was no known water-soluble vitamin. Lucerne and other legumes were among the richest sources of inhibitor. In a concentration corresponding to 0·1% of the dried plant, lucerne extracts caused more than 50% inhibition of the hydrolysis of

0·00063 M-phenolphthalein glucuronide. Inhibition by lucerne extracts was competitive, but not pH-sensitive. The active principle may have been a competing substrate or an inhibitor in the usual sense.

Liquorice root is rich in glycyrrhizinic acid, a glucuronido-glucuronide of a triterpene (Lythgoe & Trippett, 1950; Ruzicka & Jeger, 1942). In a concentration of 0·00013 M, glycyrrhizinic acid caused 50% inhibition of the hydrolysis of 0·00063 M-phenolphthalein glucuronide. Inhibition was in part competitive and in part non-competitive, and glycyrrhizinic acid itself appeared to be hydrolysed by the enzyme. Lucerne (and liquorice) preparations have, however, been obtained which on a dry weight basis were more powerful than pure ammonium glycyrrhizinate in inhibiting the hydrolysis of phenolphthalein glucuronide, and it is doubtful whether glycyrrhizinic acid, if present, could account for very much of the action of lucerne extracts.

The nature of the active principle or principles in lucerne and clovers is being investigated, with a view to studying any possible effects they may have in the animal body, in particular in animals grazed on these crops.

The ammonium glycyrrhizinate used in these experiments was presented by Dr J. W. Cornforth, to whom grateful acknowledgement is made.

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